74-12,337

SHAUGHNESSY, Peter Douglas, 1941-
BIOCHEMICAL IDENTIFICATION OF POPULATIONS OF THE HARBOR SEAL, Phoca vitulina.

University of Alaska, Ph.D., 1974
Biology-Genetics

BIOCHEMICAL IDENTIFICATION OF POPULATIONS OF THE HARBOR SEAL, *Phoca vitulina*

A

DISSERTATION

Presented to the Faculty of the

University of Alaska in Partial Fulfillment

of the Requirements

for the Degree of

DOCTOR OF PHILOSOPHY

by

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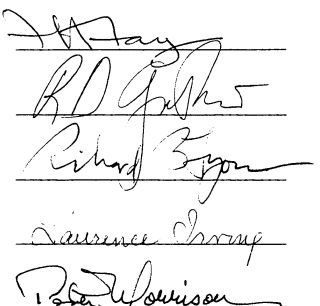
Fairbanks, Alaska

May, 1974

BIOCHEMICAL IDENTIFICATION OF POPULATIONS

OF THE HARBOR SEAL, PHOCA VITULINA

APPROVED:



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ABSTRACT

A comprehensive review of the North Pacific harbor seal literature indicated that the many names applied to these seals could be reduced to three: Phoca vitulina largha Pallas, 1811, breeding on the seasonal pack ice in Bering Sea, Sea of Okhotsk, Tartar Strait, Sea of Japan and Peter the Great Gulf; P. v. richardsi (Gray, 1864), breeding on land on the western coast of North America from Bristol Bay to Baja California, and also breeding on floating glacial ice in some regions of southeastern Alaska; and P. v. stejnegeri (Allen, 1902) (=kurilensis and insularis), breeding on land in northern Hokkaido, the Kuril Islands and eastern Kamchatka. Which of the latter two subspecies is resident in the Aleutian, Commander and Pribilof Islands is still unknown.

An electrophoretic examination of seven blood proteins controlled by 11 structural loci in 69 P. v. largha, 151 P. v. richardsi and 17 P. v. concolor detected an uncommon transferrin variant in P. v. richardsi, but failed to demonstrate any substantial difference between subspecies. Blood proteins in Phoca vitulina were less variable than those in most other organisms for which similar estimates of the levels of polymorphism and heterozygosity are available. For Phoca vitulina, the mean proportion of polymorphic loci per population was 0.02, and the mean proportion of heterozygous loci per individual was 0.001. On the basis of the observed similarity of P. v. largha and P. v. richardsi proteins, and estimates of the rate of amino acid substitutions, divergence of the two forms was estimated to have occurred less than 400,000 years ago.

ACKNOWLEDGMENTS

I wish to record my gratitude to: Dr. P. R. Morrison for his valuable advice and guidance as supervisor of this project; Dr. F. H. Fay for innumerable consultations on pinniped biology and criticism of the manuscript; Dr. L. Irving for many helpful discussions; Mr. J. J. Burns and Dr. F. H. Fay for making collections of harbor seal blood; Mr. J. J. Burns and several other officers of the Alaska Department of Fish and Game for advice in arranging collecting trips; Captain T. Roberge and the crew of USCGC Glacier who facilitated collection of material from P. v. largha in the Bering Sea; the many administrators of aquaria and biologists who permitted me to make collections from their captive harbor seals; Mr. L. Hadley of Buckland, Alaska for assistance in collecting blood samples from belugas; fellow students, Mr. J. J. Burns and Mr. E. H. Miller, for many discussions; Ms. D. Pelz for technical assistance; Mr. D. Borchert for the photographic work; Ms. C. Anderson for preparation of the typescript; and Ms. G. Shaughnessy for help in preparation of the manuscript.

Permission to collect blood samples from harbor seals in California was granted by the California Fish and Game Commission, and in Alaska by the Alaska Department of Fish and Game.

Support was provided by Sea Grant Program, Project GH83.

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I. INTRODUCTION

Alaskan marine mammals are not so well known as those from other parts of the United States, even though they are more numerous, and are of greater economic importance as a harvested renewable resource. They are also exploited commercially, especially in Bering Sea and the Sea of Okhotsk by Russian and Japanese ship-borne hunters. Reports in the Russian literature indicate extensive population studies on North Pacific marine mammals, whereas considerably less effort has been expended by the United States. There are presently no international agreements to prevent overharvesting of this valuable marine resource, with the exception of the Interim Convention on Conservation of North Pacific Fur Seals which controls the harvest of the northern fur seal, Callorhinus ursinus. Recently, an international convention formulated measures to control the expected harvest of antarctic pinnipeds (Anon., 1972), and hopefully similar discussions may focus on Bering Sea mammals. The United States is currently in an inadequate position to participate in such discussions, since it has insufficient biological information upon which to base recommendations for the wise conservation and utilization of these mammals. For effective management it is important to know which form of a particular species is present in a given area, and for this it is necessary to clarify the systematics of the group. This thesis is addressed to that problem, primarily in the harbor seal, Phoca (Phoca) vitulina, and to a lesser extent in the beluga whale, Delphinapterus leucas, and the polar bear, Ursus maritimus.

Problems of taxonomic relationships have most often been examined in terms of morphological characters which are either unweighted or, in more sophisticated analyses, combined into a multivariate statistic which takes mutual associations into account. In either case, such morphological comparisons often suffer from being based on characters that are the phenotypic expression of several genes, together with non-genetical influences. Characters much closer to the genetic material can be examined by utilizing biochemical techniques directed at the informational macromolecules (proteins and nucleic acids). However, such characters also have their limitations since they must necessarily be restricted to the present and cannot be extended to the fossil record, and fewer genes are involved for each character that is examined.

There are many techniques available for the biochemical comparison of taxa, e.g., microcomplement fixation, DNA hybridization, electrophoresis and amino acid sequencing of proteins. Each of these has its maximum utility for assessing affinities at particular taxonomic levels. For instance, microcomplement fixation is most powerful above the species level as genetic variation at or below this level is unlikely to yield antigenic differences. Electrophoresis is most useful at the lower taxonomic levels, below that of the genus, because protein polymorphisms within populations can usually be attributed to single gene substitutions, and further, such protein differences among congeneric species can be similarly attributed.

Since the development of gel electrophoresis for the demonstration of allelic variation (Smithies, 1955), several animal groups have been

studied to estimate the degree of genetic variation within populations (e.g., Harris and Hopkinson, 1972) and to compare populations of the same, or similiar, species to permit quantitative expression of genetic differences between them [e.g., Mus musculus populations by Selander, Hunt and Yang (1969)].

Such a rationale has been employed in this investigation which aims to provide estimates of genetic variation and similarity for populations of Alaskan marine mammals for comparison with estimates made from other groups whose taxonomic status has been well established.

Harbor seals are "earless" seals (family Phocidae) of the North Atlantic and North Pacific Oceans. The most recent reviews of their taxonomy are provided by Scheffer (1958) and King (1964), based on the work of Doutt (1942), who placed all of the then known harbor seals into six subspecies of Phoca vitulina:

vitulina L. in the eastern Atlantic;

concolor de Kay in the western Atlantic;

mellonae Doutt in lakes on Ungava Peninsula, Quebec;

richardii (Gray) in the eastern Pacific;

geronimensis Allen in the eastern Pacific in the vicinity of Baja California; and

largha Pallas in the western Pacific.

Scheffer and King differed from Doutt only by including P. v. geronimensis with P. v. richardii. However, Doutt could only distinguish morphologically between Atlantic and Pacific harbor seals, so that most of his separations were based on supposed geographical ranges. More recent publications

have cast doubt on this simplistic division.

Another subspecies, Phoca ochotensis kurilensis, was described from Hokkaido and the Kuril Islands by Inukai (1942). Since Phoca ochotensis Allen is a synonym of Phoca vitulina (Ognev, 1935), P. o. kurilensis should be considered as a harbor seal. It was redescribed and raised to specific status as P. insularis by Belkin (1964). However, McLaren (1966) pointed out that Inukai's name has precedence over Belkin's, and so kurilensis will be used here.

Another division of North Pacific harbor seals was proposed by Chapskii (1960) on the basis of cranial morphology and the substratum occupied during the breeding season. Those harbor seals that "haul-out" on sea ice to bear their pups have been referred to as largha, while those associated with land have been divided into richardii on the North American coast, extending north to southern Bristol Bay (Burns and Fay, 1973), and kurilensis in Hokkaido and the Kuril Islands (Belkin, 1964). The range of kurilensis was extended by Belkin, Kosygin and Panin (1969) to include eastern Kamchatka, the Commander and Pribilof Islands. Further, in an editorial footnote to that paper, it was noted that skulls of harbor seals from the Aleutian Islands also contained many characteristics of kurilensis. However, Burns and Fay (1973) included Commander and Aleutian Islands within the range of richardii, while noting similarities between specimens from the central and eastern Aleutians and kurilensis.

Thus three taxa, richardii, largha and kurilensis, are now commonly believed to occur in the North Pacific, and to inhabit Alaskan waters.

The status of these taxa is still unclear. All authors who have recently dealt with their taxonomy have recognized richardii as a subspecies of P. vitulina (Mohr, 1965; McLaren, 1966; Burns and Fay, 1973). The status of largha has fluctuated many times. Following Burns and Fay (1973), largha will be recognized as a subspecies of P. vitulina. Specific rank has frequently been accorded to P. kurilensis (=P. insularis) (Belkin, 1964; McLaren, 1966; Chapskii, 1967; Naito and Nishiwaki, 1972b). However, it has recently been reduced to a subspecies of P. vitulina by Chapskii (1969), who was impressed by the close similarity of its skulls to those of North Atlantic harbor seals. McLaren (1973) was inclined to agree with that interpretation, and it will be followed in this thesis.

Symptomatic of the confusion that has surrounded these seals is the recent superficial review by Nishiwaki (1972) in which P. v. kurilensis was not even recognized, although some of its characteristics are apparent in the description of P. v. largha.

Taxonomic nomenclature of pinnipeds in this thesis follows Scheffer (1958), with the modifications outlined by Repenning, Peterson and Hubbs (1971) for Arctocephalus. As Burns and Fay (1970) have shown, the phocines Pusa hispida, Histriophoca fasciata and Pagophilus groenlandicus are better reduced to species of Phoca; however, for convenience, generic names will be used for them in this thesis.

In this study, blood samples from P. v. largha, P. v. richardii, P. v. concolor and harbor seals of the eastern Aleutian Islands have been examined electrophoretically for 11 gene loci in order to assess

their genetic similarity, and so to clarify their systematics.

Small series of blood samples were also collected from four other Bering Sea pinnipeds (Histriophoca fasciata, Erignathus barbatus, Odobenus rosmarus and Eumetopias jubata). Affinities of these species and Phoca vitulina, based on electrophoretic comparisons of their blood proteins, have been compared with the accepted classification of pinnipeds.

Electrophoretic studies of blood proteins of beluga whales, polar bears and grizzly bears are included in appendices to this thesis. There have been few investigations of beluga populations in Alaska. Fay (1971) believed that Cook Inlet and Bristol Bay herds were resident, while other herds that apparently wintered in central Bering Sea migrated in summer into the Kuskokwim and Yukon Rivers, through Bering Strait to Kotzebue Sound, and into arctic seas. Klinkhart (1966) believed that belugas from the western Canadian Arctic and the eastern Siberian Arctic wintered in Bering Sea, and Mansfield (1971) noted that belugas from the western Canadian Arctic wintered in Bering Sea. Since material from belugas proved difficult to obtain, only one population could be studied. Some of their blood proteins have been examined electrophoretically for variation, and will provide a base against which other beluga populations can be compared in the future.

The taxonomy of polar bears has recently been examined by Manning (1971), who demonstrated the existence of a cline of increasing skull size from eastern Greenland (westward) through western Greenland-Canada and "Alaska north" to "Alaska south". The greatest difference occurred

between the last two regions, designated by a line running northwest from Point Lay. In this study series of blood samples from these two regions have been examined electrophoretically to assess population affinities. These polar bears have also been compared with a series from grizzly bears (U. arctos horribilis) from the North Slope of Alaska.

II. TAXONOMIC REVIEW OF NORTH PACIFIC HARBOR SEALS

An attempt has been made to separate the literature by taxa, but this has not always been possible, as frequently two taxa have been confounded in one description. P. v. largha will be dealt with first, as it is the most easily recognized, due to its ice-restricted breeding habitat and its lanugo-coated pups.

Although the nomenclature of North Pacific harbor seals was dealt with at some length by Allen (1880; 1902b), it seems worthwhile to reconsider several of the names in the light of present knowledge of the characteristics and distribution of the three taxa. In most early publications, descriptions were far from diagnostic and often the locality was not clearly given. However, attempts will be made to assign each of these named species to one or more of the three taxa considered here.

It will become apparent from this review that the taxonomy of largha and kurilensis has mainly been considered by Russian and Japanese workers, while that of richardii has received most attention from North American workers. Each group has, for the most part, worked in isolation from the others' material.

The localities mentioned in this review are shown in Figures 1 to 3.

II. 1. Differentiating Between the Taxa

Several attempts have been made to present diagnostic criteria for differentiating between pairs of these taxa, but all three have not been

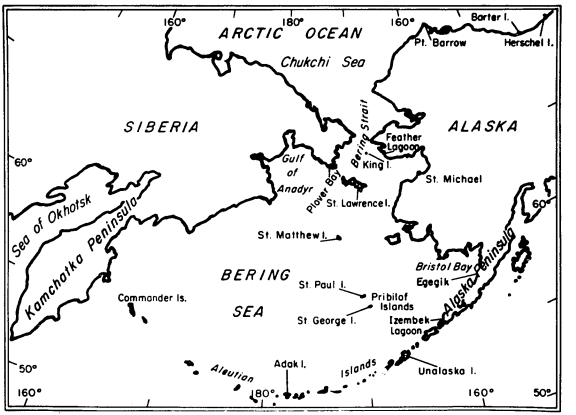

Figure 1. Map of the Bering Sea, showing places referred to in the taxonomic review of Phoca vitulina.

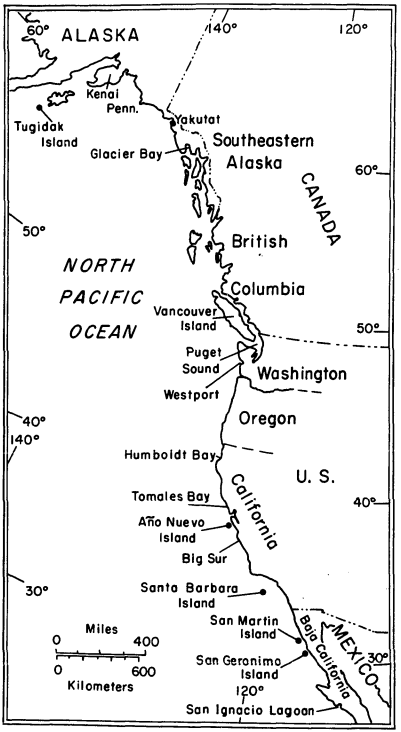

Figure 2. Map of the eastern North Pacific Ocean, showing places referred to in the taxonomic review of Phoca vitulina.

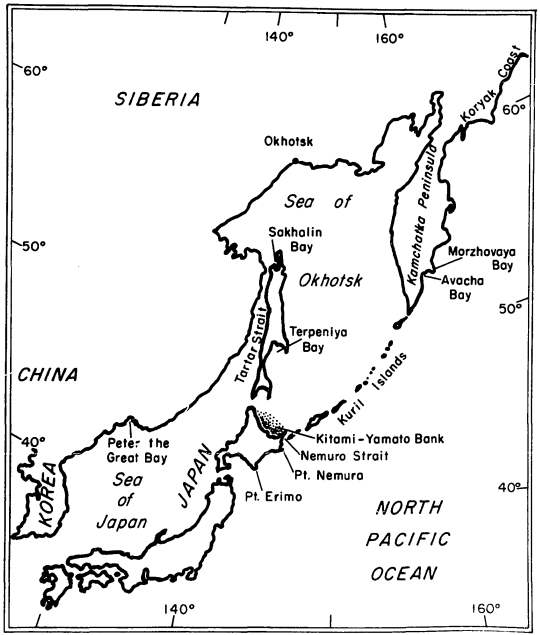

Figure 3. Map of the western North Pacific Ocean, showing places referred to in the taxonomic review of Phoca vitulina.

thoroughly considered together. Many cranial characters have been used for this purpose, but as none of these taken alone is adequate, several are required. The most recent and most successful attempt to separate largha and kurilensis is that of Chapskii (1969), who also compared them with the eastern Atlantic form, P. v. vitulina. Naito and Nishiwaki (1973) considered the two former subspecies, while Burns and Fay (1973) briefly considered all three North Pacific forms. Some of the characters in use are outlined in Table 1.

Habitat occupied during the breeding season

The character which most obviously distinguishes largha is the non-terrestrial substrate occupied during the breeding season. As largha is currently understood (Chapskii, 1960 and later; Mohr, 1965; McLaren, 1966; Naito and Nishiwaki, 1972b, 1973; Burns and Fay, 1973), it is found only on sea ice at that time. In Bering Sea it is associated with the 20- to 60-mile-wide frontal zone at the southern edge of the ice pack from Bristol Bay to the Koryak and Kamchatka coasts which consists of brash ice, small floes, pancake ice and areas of open water, and is usually referred to as the "front" (Burns and Fay, 1973). P. v. largha is also found associated with sea ice at that time in the Sea of Okhotsk, Tartar Strait, the Sea of Japan and Peter the Great Gulf (Tikhomirov, 1966a). Belkin et al. (1969) observed newborn largha on sandy beaches of the southern Kuril Islands and claimed that these observations supported Inukai's (1942) statement that largha bear their young on shore in that area. Since Inukai's observations pertained to ringed seals (fuiri) rather than to P. v. largha (zenigata), their observations should not be

TABLE 1. Comparison of characters in North Pacific harbor seals, Phoca vitulina. (For references, see text.)

Character	largha	kurilensis	richardii
Habitat during breeding season	Sea ice	Land	Land; some on floating glacial ice
Pelage in newborn pups	White lanugo	Adult-type; 2 of 22 grey lanugo	Adult-type; <1% white lanugo
Pelage in adults	Majority very pale with dark spots	Majority very dark with light rings and dark spots	Ranges from pale to dark with light rings and dark spots
Pupping time	Late March to early April (Bering Sea)	Mid-May to July	Late May to mid-July (Alaska)
Body size	Smaller	Larger	Intermediate
Skull size in adults	Majority smaller and fragile; mean of 10 adult males: 322 gm	Majority larger and massive; mean of 6 adult males: 689 gm	Majority intermediate?
Tooth size	Smaller	Larger	Intermediate to larger
Contact of premaxillaries with nasals	>3mm in 93%	<3mm in 77%	<3mm in most
Set of premolars in tooth-row	Straight in most	Oblique in most	Oblique in most
Hyoid bones	Complete	Reduced	Reduced

construed to indicate that pupping in largha does not occur on sea ice. Earlier, Belkin (1964) "observed pupping of the largha on the ice" (p. 758) in the same region.

On the other hand, the other taxa are mostly found on mud-flats, sandy beaches or rocky shores in the breeding season, with the exception that some groups of richardii in Alaska are known to haul-out on glacial ice floating on the sea, e.g., in Harris and Aialik Bays, Kenai Peninsula (Bishop, 1967, 104), and in Muir and Johns Hopkins Inlets, Glacier Bay National Monument, where they are known to breed on the ice (Streveler and Paige, 1971, 57). As there are many tidewater glaciers in southeastern Alaska, there may be many such populations of ice-inhabiting richardii.

Pelage of the newborn

Considerable attention has been paid to the pelage of newborn harbor seals. In largha it is a long, white woolly coat termed lanugo which is shed at two to four weeks of age (Tikhomirov, 1964). In richardii the white lanugo is usually shed in utero, and pups at birth have the adult-type pelage. Scheffer (1958, 94) stated that there were no records of lanugo-covered newborn richardii pups. However, there are several reports of such pups in the literature: one from British Columbia by Fisher (1952); several from British Columbia and California by Stutz (1966); several from Tugidak Island, Alaska by Bishop (1967, 98); and from the north coast of the Alaska Peninsula by Burns (1970). Both Bishop (1967) and Burns (1970) reported that such white-coats are usually born early in the breeding season and soon shed their lanugo.

Similarly, lanugo-coated pups of land-breeding P. v. concolor at Sable Island, Nova Scotia are born early in the season (Boulva, 1973). G. P. Streveler (in litt., 1972) reports that of several hundred richardii pups born annually on glacial ice in Glacier Bay National Monument, less than 1 in 100 is born with a white coat. A comparison of lanugos from a largha pup from Bering Sea and a richardii foetus from British Columbia was made by Stutz (1966) who noted the greater thickness and woollier texture of the largha pelage. He attributed this to the longer and thinner hair of largha, and its wavy nature as compared with the straight hair of richardii.

The significance of the white lanugo in richardii, largha and other pinnipeds has been discussed by Fay (1973) from a phylogenetic and physiological point of view. He considered that the ancestral, circumpolar Phocini did not have extensive contact with ice and possessed a white coat which was shed in utero. One of the harbor seal's adaptations to ice was to shift its pupping season from summer to early spring. Then molt of the white embryonal pelage, which takes place in May, occurred post-nataly in largha and in utero in most richardii.

P. v. kurilensis are also usually born with the adult-type pelage, that Belkin et al. (1969) described as black with white rings on both dorsal and ventral surfaces. However, pups with grey lanugo have been reported (Naito and Nishiwaki, 1972b). Two cases of grey lanugo in richardii pups have also been reported (Mohr, 1965), one from the Pribilof Islands, the other from Washington.

Adult pelage

Allen (1880) gave a detailed description of the highly variable color and pattern of the adult harbor seal pelage. Many subsequent authors have commented on this variation. Chapskii (1967) indicated that the common type of pelage in largha is quite pale. Bishop (1967) divided pelages of richardii from the Gulf of Alaska into two basic types: a light background with dark spots and rings; and a darker background with light spots or rings. He estimated that the light-colored type is more common, both at Tugidak Island and at Harris Bay. Pelage in kurilensis is described as being black with oval-shaped white rings (Belkin, 1964), which sounds rather like Bishop's dark type. Coat patterns of kurilensis and largha were compared by Belkin et al. (1969). Although small light-colored rings were sometimes found on the dorsal side of largha, they were not seen on its ventral side, in contrast to kurilensis. Inukai (1942) briefly described coat color in largha as "whitish-yellow with black spots", and in kurilensis as black overall with white spots. However, he also mentioned the existence of intermediate skins in the southern Kuril Islands.

Pelage color of largha and kurilensis was divided into ten classes by Naito and Nishiwaki (1973). No data were presented, but they indicated that although some overlap was observed, most largha occurred in the paler classes, and most kurilensis occurred in the darker classes. Descriptions of different authors are difficult to compare as they are so subjective. Although pelage cannot be used as a discriminating character, the impression is gained that largha has a much paler coat

than the two land-breeding taxa, and that kurilensis is usually considerably darker than the majority of richardii.

Belkin et al. (1969) also noted that the tail of kurilensis is broader than that of largha, and is marked with white stripes or rings and a dark edge, whereas that of largha is light-colored.

Pupping time

Some information on pupping time was reviewed by Bigg (1969a). This can now be amplified with more recent information, and better interpreted in terms of more modern concepts of the distribution of the taxa involved than those of Scheffer (1958) which Bigg followed.

In Bering Sea, largha has been reported by Burns et al. (1972) to pup from late March to mid-April and by Tikhomirov (1964) from early April to mid-April. There is considerable variation in time of pupping in richardii along the North American coast. In the north, along the Bering Sea coast of Alaska Peninsula, pupping occurs from late May to mid-July (Burns, 1970). As noted by Bigg (1969a), pupping generally occurs later towards the south as far as southern Puget Sound where it occurs in August and September. South of Puget Sound pupping occurs progressively earlier with decreasing latitude, until in Baja California it occurs in March, which is even earlier than in largha. Bigg did not suggest a cause for this regional variation.

The pupping season in the richardii population that reproduces on ice in Glacier Bay is similar to that of the land-breeding population studied by Bishop (1967) at Tugidak Island, i.e., from late May to mid-June (G. P. Streveler, in litt., 1972).

On the Asiatic side of the North Pacific, observations suggest a latitudinal cline in pupping dates of largha. Tikhomirov (1966a) reported that the peak occurs in mid-April in the northern part of the Sea of Okhotsk, but in mid-March in Tartar Strait and the southern part of the Sea of Okhotsk; farther south in Peter the Great Bay, pupping occurs from early February to mid-March. In the vicinity of the southern Kurils, Belkin (1964) reported that largha pupped from the end of March to mid-April, but later Belkin et al. (1969) reported that it occured from the beginning to the end of March. For the southern part of the Sea of Okhotsk, Naito and Nishiwaki (1972b) have also reported that pupping occurs in mid-March. The close relationship between time of pupping in largha in the various areas of Sea of Okhotsk and the maximum diffusion and stability of sea ice cover has been discussed by Tikhomirov (1966a).

Pupping in kurilensis takes place considerably later. On the Pacific coast of Hokkaido, Naito and Nishiwaki (1972b) reported that pups were born from the middle to the end of May. On the southern Kuril Islands, Belkin et al. (1969) noted that pupping occurred in mid-May. Earlier (Belkin, 1966), he observed that pups were born in the latter weeks of July on Sredneva Island in the central part of the Kuril Chain. On the Commander Islands, Marakov (1967) reported that pupping occurred in May and June, although earlier Barabash-Nikiforov (1935) had given late April to early May as the pupping period.

While there is some confusion about times of pupping, it is apparent that where largha and a land-breeding form are in close proximity, pupping times differ by more than a month.

Body size in adults

The adult male kurilensis evidently attains a larger body size than does either largha or richardii (Table 2).

Skulls of kurilensis were reported by Belkin et al. (1969) and by Naito and Nishiwaki (1973) to be larger and more massive than those of largha. The former authors provided data (means, standard errors and sample sizes) on weights of the skull, lower jaw and lower canine teeth in kurilensis and largha of each sex. They demonstrated differences between means of each attribute in the two subspecies using an inappropriate statistic (standard normal deviate). Values of the appropriate statistic, "Student's" t, for these comparisons based on the data presented in Table 6 of Belkin et al. (1969), are: 19.2, 15.2, and 9.8 (with 14, 15, and 13 degrees of freedom), respectively for males, and 4.2, 18.8, and 5.8 (with 19, 20, and 22 d.f.), respectively for females, all of which are significant at the 0.1% level. J. J. Burns and F. H. Fay (voc. comm.) report that richardii tends to be of intermediate size for these features.

Cranial characters

The cranial character that has received most attention for the diagnosis of North Pacific harbor seals is the extent of projection of premaxillaries posteriorly along the nasal bones and, in particular, whether or not they make contact. This was first used for the separation of Atlantic and Pacific harbor seals by True (1899) and later by Allen (1902b, 471), both attributing its first recognition to Merriam (1897). According to these authors, considerable contact is made in Pacific

TABLE 2. Body length in adult North Pacific harbor seals, Phoca vitulina.

Subspecies	Location	Body length (cm)+ ♀	♂	♀/♂	Method	Reference
kurilensis	Hokkaido	169	186	91%	Lcv*; from postnatal growth curves	Naito and Nishiwaki (1972b)
kurilensis	Kuril Islands	160.5 (18)	174.0 (17)	92%	Lcv; averages of adults	Belkin et al. (1969)
largha	Sea of Okhotsk	159.0 (8)	169.9 (14)	94%	Lcv; averages of seals over 15-y-o	Naito and Nishiwaki (1972b)
largha	Kuril Islands	145.9 (14)	150.0 (7)	97%	Lcv; averages of adults	Belkin et al. (1969)
largha	Bering Sea	143 (9)	160 (6)	89%	Lcv; averages of adults	Chapskii (1967)
largha	Sea of Okhotsk and Bering Sea	162 (27)	168 (26)	96%	Lc**; averages of seals 11-y-o and older	Tikhomirov (1968)
richardii	Vancouver and Tugidak Islands	147.7 (50)	161.1 (11)	92%	Lcv*; average of females 5-y-o and older, males 9-y-o and older	Bigg (1969b) includes Bishop (1967)

*Lcv, standard length; the straight-line distance from snout to tip of tail.

**Lc, zoological length; the shortest distance from snout to tip of tail following the curve of the dorsal surface.

+Sample size is shown in parentheses.

(Lcv is approximately 91% of Lc)

specimens, but contact is barely made, if at all, in Atlantic specimens. Doutt (1942, 114) noted that Atlantic and Pacific forms could usually be separated from each other on this basis, for in seven of his 58 Pacific specimens contact was not made, and in three of his 14 specimens from the Atlantic coast of North America, it was made. However he could not separate Pacific populations on this basis.

This feature was also used by Chapskii (1960) when he divided the Pacific population into two forms. In largha extensive contact was always made, while in the other form, slight contact was usually made. This analysis was based on small samples (only 19 Pacific specimens were mentioned). Later Chapskii (1969), using larger samples quantified this relationship: premaxillaries failed to contact the nasals in a very small proportion of largha (0.5%) and a small proportion (9%) of kurilensis, while contact was extensive (>3mm) in 93% of largha and 23% of kurilensis. Unfortunately, since Chapskii's data are expressed only as percentages with no indication of sample size, it is difficult to assess the significance of his observations. Belkin et al. (1969) noted that contact was made in each of the 54 kurilensis skulls they examined.

Chapskii (1960) also used the manner in which premolar teeth are set in the tooth-row to distinguish between Atlantic and Pacific harbor seals, and between the two Pacific populations. Later, Chapskii (1967, Table 14; 1969, Table 1) noted that teeth of adults are set straight in most largha and obliquely in most kurilensis.

Naito and Nishiwaki (1973) pointed out that premolar teeth in pups

of both largha and kurilensis are aligned obliquely and overlap one another, and that it is only in adult skulls that the difference is apparent. Burns and Fay (voc. comm.) report that teeth in richardii are also set obliquely in pups and in most adults. But Belkin et al. (1969) have confused this difference between largha and kurilensis, since they reported that kurilensis pups have premolar teeth that are set obliquely and close together, while in adults older than five years, they are almost set straight and well spaced. Conversely, their Table 5 indicates that in most pups the premolars are straight, and in most adults they are oblique. The text description fits the description of largha given by Naito and Nishiwaki (1973), while the high proportion of adults with oblique teeth in Table 5 agrees with other descriptions of kurilensis adults.

Several other cranial characters that are useful for the differentiation of largha from kurilensis have been discussed by Chapskii (1969), e.g., the length of the frontal portion of the nasals relative to their overall length, the amount of curvature of the pterygoid processes of the alisphenoid bone, the shape of the tympanic bullae, the shape of the posterior edge of the zygomatic arch, and the structure of the bone of the external auditory canal. To these, Belkin et al. (1969) have added: the profile of the skull; thickness of the rostrum; shape of the jugal bones; and shape of the posterior margin of the hard palate.

The latter authors have also noted differences between kurilensis and largha in the manner in which incomplete rings of the trachea overlap, and in the shape of the cartilages of the larynx. The most striking

osteological difference between largha and kurilensis is that the former has a complete set of hyoid bones, while in the latter, the stylohyal and tympanohyal are very much reduced (Belkin et al., 1969; Naito and Nishiwaki, 1973). At this stage there is no description in the literature of the hyoid of richardii, but Burns and Fay (voc. comm.) report that it resembles that of kurilensis rather than largha.

Naito and Nishiwaki (1973) compared skulls of 53 kurilensis with 94 largha. They commented on the well-developed sagittal crest in large kurilensis skulls, the oblique setting of their premolars, larger zygomatic breadth, mastoid breadth, rostral breadth, and greater height and breadth of the mandible.

Thus it can be seen that there are several cranial characters that distinguish largha but as yet none to differentiate between kurilensis and richardii.

II. 2. Phoca vitulina largha Pallas, 1811

Phoca largha (part) Pallas, 1811, pp. 113-114, no. 43. Eastern Kamchatka. Description includes both largha and kurilensis.

Phoca tigrina Lesson, 1827, p. 206. Kamchatka. Reference made to white pups. Absence of spots on ventral surface suggests a ringed seal.

Phoca chorisii Lesson, 1828, p. 417. Bering Strait. Based on a painting of an animal with a very pale pelage. Reference made to white pups.

Phoca nummularis Temminck, 1842, pp.3-4. Japan. Descriptions of teeth and pale pelage suggest largha.

Phoca vitulina (part) Allen, 1880, pp. 559-597.

Phoca largha (part) Nordquist, 1883.

Phoca vitulina (part) Nelson and True, 1887, pp. 264-265. Bering Sea, Chukchi Sea. White pups born on the ice in April and May.

Phoca ochotensis Allen, 1902b, pp. 480-483. Northern Sea of Okhotsk. Epithet preoccupied by Phoca ochotensis Pallas, 1811, a ringed seal.

Phoca ochotensis macrodens (part) Allen, 1902b, pp. 483-485. Specimens from Plover Bay and Barrow are within the known range of largha only.

Phoca richardii pribilofensis (part) Allen, 1902b, p. 495. Pribilof Islands. Descriptions of teeth and pale pelage suggest largha.

Phoca vitulina largha (part) Smirnov, 1908, pp. 2, 62-71.

Phoca vitulina largha (part) Smirnov, 1927a, pp. 14-15. Sea of Okhotsk, Sea of Japan, Bering Sea, Bering Strait and partly the Arctic Ocean.

Phoca vitulina largha (part) Ognev, 1935, pp. 523-541. Chukchi Sea, Bering Sea, Sea of Okhotsk, Sea of Japan, Peter the Great Gulf.

Phoca vitulina largha pallasii Naumov and Smirnov, 1936, p. 185. Sea of Okhotsk and northern Sea of Japan. Smaller in size of body, skull and teeth than P. v. l. largha.

Phoca petersi Mohr, 1941, p. 58. Korea; also specimens from Hokkaido and Alaska Peninsula. Largha according to Schwarz (1942).

Phoca vitulina largha (part) Doutt, 1942, pp. 117, 122. Asiatic side of the North Pacific Ocean.

Phoca vitulina richardii (part) Doutt, 1942, pp. 117, 120-121. American side of the North Pacific Ocean [portion].

Phoca largha (part) Chapskii, 1955, pp. 177-185. Typical largha especially evident in the Sea of Okhotsk.

Phoca vitulina largha (part) Scheffer, 1958, pp. 93-95. From Bering Strait southwestward along Asiatic shores and islands to China; northwestward into the Chukchi Sea.

Phoca vitulina richardi (part) Scheffer, 1958, pp. 92-93. North coast of North America, from Herschel Island to eastern Bering Sea.

Phoca vitulina largha Chapskii, 1960, pp. 355-358. Southern Chukchi Sea, east to Point Barrow or Herschel Island; Bering Sea and Sea of Okhotsk. Pagophilic.

Phoca vitulina largha (part) King, 1964, pp. 52, 55, Map 19. Bering Sea on Asiatic coast, Kuril Islands, Sea of Okhotsk, Sakhalin, Tartar Strait, Hokkaido [north coast], as far south as Shantung Peninsula. Pups born on ice floes.

Phoca vitulina richardi (part) King, 1964, pp. 52, 55, Map 19. Herschel Island, Alaskan coast [portion], eastern Bering Sea.

Phoca vitulina largha Mohr, 1965, pp. 273-287, Fig. 7. Chukchi Sea east to Herschel Island, Alaskan and Siberian coasts of Bering Sea, Sea of Okhotsk, Tartar Strait, Sea of Japan, Yellow Sea.

Phoca largha McLaren, 1966, p. 467. Pagophilic.

Phoca largha Chapskii, 1967, pp. 147-176. Bering Sea, Sea of Okhotsk. Pagetoda form.

Phoca vitulina largha Belkin, Kosygin and Panin, 1969, pp. 157-175. Kuril Islands.

Phoca largha Chapskii, 1969, pp. 294-304.

Phoca vitulina largha Naito and Nishiwaki, 1972b. Pagophilic. Compared largha and stejnegeri in the southern Sea of Okhotsk.

Phoca vitulina largha (part) Nishiwaki, 1972, pp. 171-173. From Bering Strait to coast of China, Sea of Okhotsk.

Phoca vitulina richardi (part) Nishiwaki, 1972, pp. 171-172. Herschel Island to eastern Bering Sea.

Phoca vitulina largha Burns and Fay, 1973, p. 48. Bering Sea; occur in winter and spring in "front" zone of southern edge of ice pack from northern Bristol Bay to Koryak and Kamchatka coasts.

Early descriptions

North Pacific seals were divided by Steller (1751) into three varieties on the basis of size, one of which was probably the harbor seal: "Those of medium size are all as large as a tiger, and are marked with many smaller spots" (p. 180). Later, (Steller, 1774, 107) he was clearly referring to the harbor seal: "they have over the entire body spots of equal size like a tiger which are sometimes chestnut brown or black, but the background is always yellow-white, and a few are totally white". He noted that the young have "hair as white as snow", clearly indicating largha.

Largha is an Okhotsk name for harbor seal according to Ognev (1935, 524). It was first used by Pallas (1811, 113, no. 43) for seals of the eastern coast of Kamchatka. However his description appears to include both kurilensis and largha (as it is presently known). Also, the type locality is probably within the range of both forms, and certainly within the range of kurilensis (Belkin et al., 1969). Pallas stated: "pups born on the shore immediately follow their mother", and noted that the seal was called "tschernaja nerpa" (black seal) by the Russians, both

of which certainly refer to kurilensis. However, most of his description of pelage is indicative of largha: "the body on the upper side is shiny white, sprinkled with black oval-shaped spots", and "Color greyish-white, shiny, sprinkled with oblong-oval shaped black spots, more abundantly on the back, and these are interspersed with less dark ones so that the back becomes almost black". The remainder of Pallas' description is of internal organs and is not useful in diagnosis. Thus the original largha of Pallas seems to have included two of the currently recognized taxa: largha in the sense of Chapskii (1960) and Burns and Fay (1973); and kurilensis (=insularis) in the sense of Belkin (1964) and Naito and Nishiwaki (1972b). In such a situation, the name of the subdivided taxon must be retained for one of the components (International Commission on Zoological Nomenclature, 1964, Article 47a). In this case, the original name should be retained for the ice-breeding component, as it has been used in this sense since Chapskii (1960) introduced the restricted, ice-breeding concept for largha.

The next author to mention North Pacific harbor seals was Choris (1822). Although he did not apply a scientific name, his water color (Plate 8) of a "chien de mer du détroit de Behring" obviously depicts largha judging by the very pale pelage and the locality. Lesson (1828, 417) named P. chorisii on the basis of Choris' description, and stated that the "young are white as snow". He also described varieties from the Aleutian Islands ("dirty white without spots") and the Kuril Islands ("black, spotted with white"), but it is not clear that they represent harbor seals. Earlier (Lesson, 1827), he had described Phoca tigrina

from Kamchatka on the basis of a description of the Pacific harbor seal by the Russian traveller Krasheninnikov (1768), who referred to it as "phoque tigré" (striped seal). Allen (1880, 584) dismissed both chorisii and tigrina because they were "based on the vague descriptions of travellers or unscientific writers", but it seems clear now that chorisii could only have been the ice-breeding largha. P. tigrina has been considered as a harbor seal by Allen (1880), Smirnov (1927a) and Scheffer (1958), and so becomes a synonym of P. v. largha on the basis of its white young. But Lesson's brief description is confusing, for although the presence of spots on the dorsal surface suggests a harbor seal, their absence from the ventral surface suggests a ringed seal (Allen, 1880, 562 and 600).

Another early description of North Pacific harbor seals is that of Temminck (1842) in which he named Phoca nummularis to include P. largha as a junior synonym. This work is notable in that it includes the first mention of craniological comparisons with other phocids, especially the ringed seal. Temminck had portions of the skulls of three young individuals as well as skins from these, and from three adults, all from Japan. Later, Gray (1864) also noted, after examining Temminck's cranial material, that nummularis was similar to (although distinct from) Pagomys foetida (i.e., ringed seal) of the same age, but with a thicker jaw and heavier grinding teeth. Allen (1880, 579) pointed out that neither Temminck nor Gray had compared nummularis specimens with those of Phoca vitulina, and decided that the two were identical. However, he later contended (Allen, 1902b, 466) that nummularis was

"similar in coloration to Phoca foetida but smaller and with heavier dentition -- features which may characterize a species of seal found in Japan, and still practically unknown". Subsequently, Smirnov (1927a), Ognev (1935) and Ellerman and Morrison-Scott (1966) accepted nummularis as a harbor seal without comment. Temminck's description of heavier dentition and thicker jaw is reminiscent of kurilensis; conversely he mentioned that "the dental system...does not differ from that of the crescent [harp] seal and the ringed seal" (p. 3), which species have teeth more like those of largha than kurilensis, in that they are set straight in the tooth-row and are relatively small. Furthermore, his description of the pelage as rather pale, and more particularly, of one of the skins as "completely similar to one depicted by Choris" (p. 4), indicates that Temminck's nummularis was largha as it is presently known.

Allen's taxonomic revision

Several new taxa of North Pacific harbor seals were named in an extensive revision by Allen (1902b): P. ochotensis from the northern part of the Sea of Okhotsk; P. o. macrodens from "southeastern Kamchatka north to Point Barrow" (p. 484); P. stejnegeri from the Commander Islands and neighboring coast of Kamchatka; P. richardii pribilofensis from the Pribilof Islands; and P. r. geronimensis from San Geronimo Island, Baja California. His P. ochotensis, on the basis of its cranial and dental characters, certainly represents present-day largha. Furthermore, as Ognev (1935) pointed out, this epithet had been preoccupied by a ringed seal described by Pallas (1811) under the name Phoca ochotensis. Both Ognev (1935) and Scheffer (1958) used ochotensis as a subspecific name

of the Okhotsk ringed seal, Pusa hispida ochotensis. The P. o. macrodens of Allen, judging by its large teeth, some of which were set obliquely in the tooth-row, and its type locality in Avacha Bay, Kamchatka, was appparently kurilensis although the skull described in detail from Plover Bay is a good example of largha.

Of P. stejnegeri, Allen wrote that it was "similar in general features to Phoca vitulina [the Atlantic harbor seal] but much larger, and differing essentially in cranial and dental characters" (p. 486). He stated that its skull was "fully twice as large as that of Phoca vitulina" (p. 488), although his data do not substantiate this. After comparing skulls and teeth of P. stejnegeri in Allen's description with P. kurilensis material, Chapskii (1969) and Naito and Nishiwaki (1972a) concluded that they were synonyms on the basis of large skull size, oblique setting of the premolars and locality. Stejneger (1896) and Barabash-Nikiforov (1938) also wrote about hair seals of the Commander Islands using the name largha. Recently, the ecology of these seals has been discussed by Marakov (1967). He described them as a large-bodied, resident form with a dark pelage, and assumed that they were related to kurilensis. In an editorial footnote Chapskii assures us that this is so. Marakov (1967) noted that Barabash-Nikiforov (1935) wrote of both settled and migrant seals at the Commander Islands, indicating that largha also occur there. However, Marakov did not observe the latter ashore.

P. r. pribilofensis was described as a new subspecies "on the basis especially of the three Pribilof Island skulls" (p. 495). Judging by Allen's description of the teeth ["small and non-obliquely set" (p. 493)

and "decidedly and uniformly weaker in Alaska specimens than in those from Puget Sound" (p. 495)] and the pale pelage, the Pribilof specimens were largha from the nearby Bering Sea ice pack. Since the two other specimens were taken beyond the present known range of largha (Adak Island and Yakutat), they were probably not largha, but richardii.

Seals on the Pribilof Islands received much attention from biologists at the turn of the century, but most of this was directed toward the northern fur seal, Callorhinus ursinus, and little attention was given to harbor seals. Elliott (1881) briefly discussed the hauling-out places (wave-washed rocks) of "Phoca vitulina" and its lanugo-covered pups which weighed from three to seven pounds. The description of the pup's pelage is suggestive of largha, but the weights are very light for harbor seals. A series of 48 largha pups, including some newborn from the vicinity of the Pribilof Islands, weighed from 16.5 to 69.4 lb (7.5 to 31.5 kg) (Burns et al., 1972), and six newborn richardii puns from Tugidak Island averaged 26 lb (11.8 kg) (Bishop, 1967, Table 12). The latter author also reviewed earlier data on weights of newborn pups from other localities which were similar to the Tugidak sample. The pups described by Elliott were more likely to have been ringed seals which weigh about 4 kg (8.9 lb) at birth (Burns, 1970).

Pribilof Island harbor seals were referred to as Phoca largha by True (1899) who cited Merriam (1897) as his authority. Merriam based his decision on the number of cusps on the premolars and molars (not considered to be diagnostic by Chapskii, 1969), and on the extensive contact of the premaxillaries with the nasals, which is indicative of

largha but also occurs in *richardii* and *kurilensis* to some extent. Harbor seals were not even mentioned by Osgood, Preble and Parker (1914), nor by Hanna (1923) in their accounts of Pribilof fauna. Preble (1923) referred Pribilof Island harbor seals to *P. r. pribilofensis* (=*largha*), but stated that they "have their young on the bare sea-washed rocks" which indicates that they were not *largha*.

More recently, Scheffer (in Mohr, 1965) noted that "Apparently the Pribilofs have a resident population of *P. v. richardi* [i.e., land-breeding harbor seals] and a migrant, small and very erratic visitation of *P. v. largha* [i.e., ice-breeding harbor seals]." Observation of 20 nursing pups on St. George Island by T. C. Newby (*in litt.*, 1972) on 15 July 1971 is further support for the presence of a land-breeding population.

Although the early literature suggests that the harbor seal of the Pribilofs was *largha*, all later evidence indicates the presence of a land-breeding form, which has often been assumed to be *richardii*. But the situation has been complicated by the inclusion of the Pribilofs in the range of *kurilensis* by Belkin *et al.* (1969). Support for this conclusion is provided by both Ognev (1935, 533) and Chapskii (1969). The former could not find any cranial differences between skulls from the Commander Islands, on the one hand, and at least two skulls from the Pribilofs and one from Unalaska Island, on the other. The latter author examined two skulls from the Pribilofs and decided that they were *kurilensis*. Thus the Pribilofs are located in the general area where distributions of the three taxa are currently thought to overlap. It is unfortunate that so little is known of the harbor seal there, especially as the locality

has been frequented by pinniped biologists for many years in connection with the harvest of northern fur seals.

Development of the concept of two North Pacific taxa

For many years Russian authors referred to all North Pacific harbor seals as either P. largha (Nordquist, 1883; Chapskii, 1955), or P. v. largha (Smirnov, 1908 and 1927a; Ognev, 1935). However, Ognev (1935, 532) noted some morphological heterogeneity, in particular that skulls of northern Okhotsk seals were generally smaller than those from the Commander Islands and the east coast of Kamchatka. He went on to state that if these features are confirmed, "the North Okhotsk largha will have to be recognized as a distinct natio [i.e., race], P. v. largha, natio nova". Naumov and Smirnov (1936) extended this concept and divided North Pacific harbor seals (which they treated as a subspecies of P. vitulina) into two "nationes" on the basis of new cranial material from the Sea of Okhotsk (22 skulls) and Bering Sea (19 skulls). They referred to harbor seals from the Sea of Okhotsk and northern Sea of Japan as P. v. largha pallasii natio nova, and those from Bering Sea and adjacent parts of the Pacific Ocean as P. v. l. largha Pallas. The older name, largha, given by Pallas, was retained for Bering Sea animals on the basis of priority. The pallasii animals were smaller in body, skull and teeth than the quadrinomial largha. From their descriptions of the two forms it is clear that we would now include pallasii in the present day concept of largha, and their quadrinomial largha in kurilensis.

Only a single form of North Pacific harbor seal was recognized by Chapskii (1955, 177), although he was aware that these animals showed

much more cranial variation than did those of the Atlantic. Surprisingly, he made only slight reference to the perspicacious work of his mentor, Smirnov, outlined above (Naumov and Smirnov, 1936). In a later publication, Chapskii (1960) demonstrated how the Pacific harbor seal differed from the type subspecies of the Atlantic, and pointed out that two distinct morphological and ecological types could be observed among Pacific animals. One of these, from the western part of the range, especially the Sea of Okhotsk, was referred to as the "largha-like" form, and was considered to be pagophilic, meaning (to quote Chapskii, 1960, 345) "a tendency to use ice for reproduction and molt". The other form, from the Aleutian and Commander Islands and coast of Kamchatka, was considered to be less homogeneous, and to be pagophobic [i.e., "not bound to the ice, reproducing on dry land" (Chapskii, 1960, 347)]. These two forms are now recognized as largha and kurilensis, respectively. Chapskii suggested that the latter form resembled the subspecies P. v. richardii.

According to Chapskii (1960) the term "pagophilic" was introduced by Smirnov (1927b). It is used in the same sense as "pagetoda", defined by Chapskii (1967, 149) as "restriction to ice during the reproductive period". Pagophobic is used synonymously with "egialodic", another term which, according to Chapskii (1969), Smirnov (1927b) introduced. It is a Greek word meaning "shore" (Chapskii, 1967, 171). The first two of these terms (pagophilic and pagetoda) are frequently used to refer to largha, while the others (pagophobic and egialodic) are often used to refer to richardii and kurilensis. However, as it seems that a large portion of the richardii population utilizes glacial ice during the

breeding season, these terms are equivocal, and so should be used with caution or not at all. This also applies to the terms "ice-breeding" and "land-breeding".

North Pacific harbor seals were also divided into two taxa by Doutt (1942), in this case simply on the basis of location: P. v. largha for the Asiatic side and P. v. richardii for the American side. However, his conclusions were quite unwarranted, as he could not present any diagnostic characters for their recognition. Doutt's material contained only seven specimens listed as "largha". Four of these were from the northern Sea of Okhotsk, one was from the Gulf of Anadyr, and a sixth was from north-eastern Siberia. On the basis of location, these are most likely to have been largha. The final specimen, from Avacha Bay, Kamchatka, had been used by Allen (1902b) to describe P. o. macrodens, and was probably kurilensis, as presently known (see page 30). Judging from the location of Doutt's 85 "richardii" specimens, at least nine were probably largha, for these were taken in localities north of the present known range of richardii (viz., St. Lawrence Island, King Island, Point Barrow and St. Michael). Thus, it is little wonder that Doutt was unable to differentiate morphologically between the two taxa he recognized.

The weakness of Doutt's analysis was perpetuated by later authors (Scheffer, 1958; Davies, 1958; King, 1964; Nishiwaki, 1972) who gave as the geographical separation between the two taxa, to quote Scheffer (1958, 90), "wide expanses of open water". This concept was severely criticized by Chapskii (1960) who claimed that the open sea was no barrier at all to these seals. Rather, he believed, their distribution is related to the

distribution of sea ice. Thus, largha is found in the southern part of Chukchi Sea, east as far as Point Barrow, or beyond, and in the Bering Sea east as far as the coast of Alaska. On the other hand, "the non-'pagophilic' form (P. v. richardi) is probably distributed to the western limit of the Bering Sea" (p. 358). Further, he suggested, the two forms overlap in Bering Sea, especially during the ice-free summer and autumn. Even now it is not known to what extent this occurs. A distribution map of these two forms was shown by Mohr (1965).

At about the time that Doutt (1942) suggested the one taxon-one coast concept which became so popular, Schwarz (1942) proposed that all of the Bering Sea should be included in the range of largha, and that the range of richardii extended along the North American coast as far north as Alaska Peninsula. It is unfortunate that Schwarz did not elaborate on how he made this distinction, for his ideas of range of the two taxa were closer to current views than were those of most of his contemporaries.

Another species of North Pacific harbor seal, P. petersi, was described about this time by Mohr (1941). It was named from two immature specimens obtained in Korea, one skull from Hokkaido, and another skull from Izembek Lagoon, Alaska Peninsula. This species was quickly refuted by Schwarz (1942) who decided that the cranial characteristics noted by Mohr could also be observed in largha. Nor did Chapskii (1955, 184) recognize P. petersi. Presumably Mohr was prompted to recognize her specimens as a new species because Korea was then beyond the well-known range of largha (Ognev, 1935, 533), although harbor seals had already been reported farther south by Allen (1938) and Leroy (1940). Its occurrence in Korea has been

recorded by Van Hon Gu (1956). P. v. largha breeds in Peter the Great Gulf (Tikhomirov, 1966a), and presumably it is these seals that range southward to Korea and the coast of China. In a later publication Mohr (1965) decided that petersi was a junior synonym of largha.

The ice-breeding habit

It is now known that largha breeds on sea ice (Chapskii, 1967; Burns and Fay, 1973). The earliest reference to the ice-breeding habit appears to be in a description of the harbor seal in Alaskan waters by Nelson and True (1887). They state quite explicitly that the young, which are covered with a "thick coat of slightly curly or crinkled white hair" are born "upon the ice during April and May" (p. 264) in the area north of the Pribilof Islands. This report seems to have been overlooked for there is no further reference in the English literature to harbor seals breeding on ice until Wilke (1954). In the Russian literature the ice-breeding habit seems to have been known for some pinnipeds, and possibly harbor seals, since 1927 when [according to Chapskii (1960)] Smirnov (1927b) introduced the term "pagophilic" and its antithesis, "egialodic". More direct information was provided by Ognev (1935, 538) who quoted a letter from V. S. Stakhanov stating that in Sakhalin Bay, parturition takes place on ice floes in February and March, and that pups are born with a white lanugo. Later, Chapskii (1955, 184) referred to the ice-breeding habit of North Pacific harbor seals when distinguishing between them and the Atlantic form.

Sea of Okhotsk largha

Ice-breeding harbor seals in the southern part of Sea of Okhotsk

were referred to by Inukai (1942) and Wilke (1954). The former wrote of "gomafu-azarishi" (harbor seals) which are migratory and gather on the ice of Taraika-wan (Terpeniya Bay), Sakhalin, for breeding. During the summer, he reported, they are common on the Okhotsk coast of Hokkaido, on the southern Kurils and on the Pacific coast off Point Nemuro. Their presence at this last location was attributed to the cold south-flowing current.

Wilke (1954) noted the presence of a harbor seal that produced a lanugo-covered pup on ice floes from February to March in the southern Sea of Okhotsk. After examining skulls of this form, he decided that they were a geographic race of P. vitulina, and not of the ringed seal, Pusa hispida.

The distribution of largha in Sea of Okhotsk from April through October was described by Tikhomirov (1961; 1966b). In most localities they are sedentary, hauling-out on shore in summer, and spending winter on offshore sea ice. But those that winter off the northern coast of Hokkaido bear their pups there in April or earlier, migrate northward along the coast of Sakhalin and form rookeries on the western shore of the Sea of Okhotsk in July.

Belkin (1964) reported that largha is distributed in the northern and southern Kurils, where it is sympatric with kurilensis (=insularis). However, Belkin thought that they were reproductively isolated, due to the difference in pupping dates. Although he noted that largha bears its pups on sea ice in that area, he stated that both forms lead a settled mode of life, suggesting that largha does not travel far from the ice to

its summer hauling-out grounds on the islands.

Chapskii (1967) collected a large series of skulls from harbor seals on Bering Sea ice during the breeding season, and could not find any significant differences between them and those from Sea of Okhotsk. Further, he considered largha and kurilensis skulls to be morphologically distinct, and so raised largha to specific status to match that which had been accorded kurilensis by Belkin (1964). However, he pointed out that he was still unsure of their relationships to richardii.

Naito and Nishiwaki (1972b; 1973) studied largha in the southern part of the Sea of Okhotsk, and on the northern and eastern coasts of Hokkaido; they also studied kurilensis on the east coast of Hokkaido, and reported on the life history, growth, distribution, pelage and cranial characters of both taxa. They found largha on the ice pack of the Sea of Okhotsk from January to April, and in lagoons on the north coast of Hokkaido during the summer. In autumn some occurred on the Pacific coast of Hokkaido (i.e., within the range of kurilensis). Naito (1971) has described hunting of largha in the Sea of Okhotsk on ice floes above the Kitami and Yamato Seamount and, to a lesser extent, in the deeper waters of Nemuro Strait, and in lagoons on the northern coast of Hokkaido. Tikhomirov (1966b) has described methods used to hunt largha at shore rookeries in the Sea of Okhotsk during summer and autumn.

Bering Sea largha

Tikhomirov and Kosygin (1966) noted that largha pairs form on the ice in March, as much as two months before mating. Burns et al. (1972) observed that in early April family groups consisting of a mother-pup

pair and an adult male were rarely closer than 0.25 km to one another. This contrasts with the more gregarious nature of both kurilensis (Naito and Nishiwaki, 1972a) and richardii (Scheffer and Slipp, 1944; Bishop, 1967). Pupping begins in late March (Burns and Fay, 1973), reaching a peak in early April, and most matings take place in early May (Tikhomirov, 1964). The latter author also reported that molting of the young from their lanugo to the adult-type coat begins in late April, and weaning takes place from late April to the middle of May. These seals migrate passively northward with drifting ice during the period when pups are being born and are molting (Tikhomirov, 1964). During summer they are known to occur on the Alaskan coasts of Bering, Chukchi and Beaufort Seas, at least from St. Lawrence Island to Herschel Island. In autumn they return to Bering Sea with the southward advance of the ice (Burns and Fay, 1973).

Taxonomic status of largha

The taxonomic status of largha is still unclear. Early Russian authors (e.g., Smirnov, 1908 and 1927a; Ognev, 1935; Naumov and Smirnov, 1936) considered it to be a subspecies of P. vitulina. Chapskii (1955) accorded it specific rank as P. largha, recognizing differences between Atlantic and Pacific animals in several cranial characters, habitat occupied during the breeding season, and coat color of pups. Later (Chapskii, 1960), he extended these views, recognized the existence of another form of harbor seal in the North Pacific (the land-breeding P. v. richardii), and accorded largha subspecific status as P. v. largha. Finally (Chapskii, 1967), influenced by differences between kurilensis

(which he recognized at the specific level) and largha, he returned the latter to specific rank, and has since maintained that attitude (Chapskii, 1969). Most other recent authors, however, have accorded it subspecific rank: Belkin (1964); Mohr (1965); Naito and Nishiwaki (1972b) and Burns and Fay (1973), although McLaren (1966; 1973) recognized it as a full species. It has been recognized as a subspecies here, following Burns and Fay (1973), since it is not yet clear whether it is reproductively isolated throughout its range.

II. 3. Phoca vitulina stejnegeri (Allen, 1902)

Phoca largha (part) Pallas, 1811, p. 113, no. 43. Eastern Kamchatka. The "black seal" with "pups born on the shore".

Phoca ochotensis macrodens (part) Allen, 1902b, pp. 483-485. Large teeth, some obliquely set, suggest stejnegeri. The type and one other specimen, both from eastern Kamchatka, are from within the known range of stejnegeri.

Phoca stejnegeri Allen, 1902b, pp. 485-491. Commander Islands and neighboring coast of Kamchatka. Large skull and oblique setting of premolars suggest stejnegeri (Chapskii, 1969; Naito and Nishiwaki, 1972a).

Phoca vitulina largha (part) Smirnov, 1927a, pp. 14-15. P. stejnegeri included in synonymy.

Phoca vitulina largha largha Naumov and Smirnov, 1936, p. 186. Bering Sea and adjacent parts of the North Pacific Ocean. Larger body, skull and teeth than P. v. l. pallasii.

Phoca ochotensis kurilensis Inukai, 1942, p. 930. Pacific coasts of Hokkaido and Kuril Islands. Dark pelage.

Phoca vitulina largha (part) Doutt, 1942, pp. 117, 122. Asiatic side of the North Pacific Ocean [portion].

Phoca largha (part) Chapskii, 1955, pp. 182-183. Morphological deviations from typical largha occur most frequently in the Aleutian Islands.

Phoca vitulina largha Scheffer, 1956, p. 352. Comments on Inukai's (1942) description of P. ochotensis kurilensis.

Phoca vitulina largha (part) Scheffer, 1958, pp. 92-94. From Bering Strait southward along Asiatic shores and islands [portion].

Phoca vitulina richardi (part) Chapskii, 1960, pp. 355-358. Commander Islands, coast of Kamchatka. Pagophobic.

Phoca insularis Belkin, 1964, pp. 1217-1219. Kuril Islands. Dark pelage, massive skull, pups born with adult-type pelage.

Phoca vitulina largha (part) King, 1964, pp. 52, 55, Map 19. Commander and Kuril Islands, [eastern] Kamchatka, [eastern] Hokkaido.

Phoca vitulina richardi (part) Mohr, 1965, pp. 273-287, Fig. 7. Commander Islands, southeastern coast of Kamchatka, eastern coast of Kuril Islands, and eastern coast of Hokkaido.

Phoca kurilensis McLaren, 1966, pp. 470-471.

Phoca insularis Chapskii, 1967, pp. 147-176. Kuril, Commander, Aleutian and Pribilof Islands and eastern Kamchatka. The egialodic or pagophobic form.

Phoca insularis Belkin, Kosygin and Panin, 1969, pp. 157-175. Kuril and Commander Islands, eastern Kamchatka.

Phoca vitulina insularis Chapskii, 1969, pp. 294-304. A single species of pagophobic harbor seal in the North Pacific Ocean.

Phoca kurilensis Naito and Nishiwaki, 1972b, pp. 127-144. East coast of Hokkaido and Nemuro Strait. Pagophobic.

Phoca vitulina largha (part) Nishiwaki, 1972, pp. 171-173. Kuril Islands, Commander Islands, western shore of Bering Sea [portion].

Early descriptions

The land-breeding harbor seal from the western North Pacific was described by Inukai (1942, 930) from Hokkaido and the Kuril Islands, and given the name P. ochotensis kurilensis. He also referred to it by the Japanese name, "zenigata-azarishi", as distinct from the ice-breeding harbor seal, "gomafu-azarishi" (i.e., largha), and noted that "people call it 'kuro' - black" due to its dark-colored pelage which contrasts with the paler "gomafu". However, intermediate pelages were also said to exist, which suggests that there must have been some overlap in coloration between the two types. He also indicated that it is a coastal-dwelling, non-migratory seal, whereas the "gomafu" is migratory. Although Inukai took no measurements of this seal, and despite the vagueness of his description, there is little doubt that he was indeed describing what is now known as kurilensis or insularis and was described by Allen (1902) as P. stejnegeri. However, some of Inukai's statements are ambiguous; for example, he stated that "body size is almost the same as 'gomafu'", and "many of them are rather small", and

further, that "their skulls resemble those of 'gomafu'". Body length measurements of largha and kurilensis show, on the contrary, that kurilensis attains a greater length than does largha (see Table 2). Also, Belkin (1964), Belkin et al. (1969) and Naito and Nishiwaki (1973) have commented on the massiveness of skulls of kurilensis in contrast with those of largha. Presumably Inukai did not have large adults when he made his observations. Also ambiguous is Inukai's statement concerning the pelage of the newborn which he gives as "yellowish-white long, downy hair". Presumably Scheffer (1956) was influenced by that statement when he included kurilensis in the synonymy of largha. Conversely, Belkin (1964) and Belkin et al. (1969) found pups still with an umbilical cord and yet with the adult-type pelage, and assumed that they were born with this coat. Naito and Nishiwaki (1972b) observed 20 pups with umbilical cords, all of which had the adult-type pelage, and two others with a lanugo coat that was not white, but grey. Unlike lanugo-covered richardii pups which are usually born early in the pupping season (see page 14), these two grey pups were born in the middle of the season. They also noted that fishermen had reported to them the occurrence of near-term foetuses with the same grey lanugo. The significance of this previously unreported lanugo was not discussed.

In their brief outline of Japanese seals, Nishiwaki and Nagasaki (1960) mentioned kurilensis, but considered its skull to be very similar to that of largha, and did not recognize it as a separate taxon. Otherwise, kurilensis was overlooked until it was redescribed by Belkin (1964). Even though he referred to Inukai's work, Belkin, for no apparent reason,

gave this seal a new name, P. insularis. On the other hand, McLaren (1966) used kurilensis pointing out that Inukai's name had precedence over Belkin's. Naito and Nishiwaki (1972a) noted further that the cranial and dental characters of the Kuril seal resemble those of P. stejnegeri described by Allen (1902b) from "Bering Island and neighboring points on the Kamschatkan coast" (p. 486), and concluded that the latter should be included in the synonymy of P. kurilensis. Rather, it would seem that stejnegeri is the senior synonym in this case and should take precedence over kurilensis. Earlier (p. 30) it was noted that P. ochotensis macrodens Allen (1902b) is also identical with kurilensis. As macrodens and stejnegeri were published simultaneously, the question arises as to which name should take priority. Allen's description of stejnegeri contains much more detail than that of macrodens, and the type of stejnegeri is an old adult while that of macrodens is merely a young adult. Further, specimens of stejnegeri were from the Commander Islands and the southeastern coast of Kamchatka, localities where "kurilensis" breeds and largha visits infrequently whereas, only the type of macrodens and another specimen were from a locality which is now included in the range of "kurilensis" (eastern Kamchatka). The other five specimens of macrodens were from locations currently considered to be in the range of largha alone (Plover Bay and Point Barrow). Thus, following Recommendation 24A of the International Commission on Zoological Nomenclature (1964), stejnegeri should take priority, even though macrodens has page precedence, and the epithet should be "stejnegeri (Allen, 1902)".

Distribution

According to Inukai (1942) this seal is found on the Pacific coasts of Hokkaido (from Point Erimo northeastward) and the Kuril Islands. Naito and Nishiwaki (1972a) gave more precise information on hauling-out grounds in Hokkaido. On the basis of fishermen's reports, they estimated the population size to be about 500. They also indicated (Naito and Nishiwaki, 1973) that some stejnegeri are seen in the Sea of Okhotsk. Belkin (1964) counted 1660 stejnegeri (excluding pups) throughout the Kuril Chain, and estimated that the population barely exceeded 2000-2500. Their distribution in the Kuril Islands was briefly described by Belkin et al. (1969), and reference was made to a more detailed description (Belkin, in press). Belkin et al. (1969) noted that stejnegeri occurs in the Commander Islands and on the eastern coast of Kamchatka at Morzhovaya Bay. They also included the Pribilof Islands in its range, on the basis of the skull of a large adult male in the collection of the Zoological Institute of the Academy of Sciences of the USSR. In an editorial footnote, it was noted that skulls in that collection from the Aleutian Islands (as well as the Commander Islands and eastern Kamchatka) also contained many characteristics of stejnegeri. Marakov (1967) estimated the population on the Commander Islands to be 1500. Harbor seals are reported as common, although not abundant, throughout the Aleutian Islands (Murie, 1959). Consequently, the population of stejnegeri must be considerably greater than estimated earlier. In the IUCN Red Data Book (Simon, 1967) its status (as "kurilensis") was given as "indeterminate, apparently in danger", but in the light of

more recent information, it would seem that this should be revised.

The population of stejnegeri can be divided into two groups according to their association with sea ice in the same way as Fay (1973) divided the richardii population of Bering Sea (see page 54). Those of the Kuril Chain and the eastern Kamchatkan coast have extensive contact with ice, while those of northern Hokkaido, the Commander and Aleutian Islands have little or no contact.

Sexual dimorphism

Sexual dimorphism in body size of stejnegeri has been reported by Naito and Nishiwaki (1972b) and by Belkin et al. (1969). They found that females attained 91% and 92%, respectively, of the standard length (Lcv) of males (see Table 2). The difference expressed by the latter percentage is significant ($t_{23} = 2.87$, $.001<P<.01$). For largha, the corresponding figures of these authors were 94% and 97%, of which only the former represents a significant difference between sexes ($t_{20} = 2.14$, $.02<P<.05$; and $t_{19} = 1.00$, $.30<P<.40$). Chapskii (1967) also found sexual dimorphism in a sample of largha from the Bering Sea, in which females attained only 89% of the standard length of males. On the other hand, Tikhomirov (1968) found little difference in the zoological length (Lc) of males and females from the Sea of Okhotsk and Bering Sea. These figures for largha are not directly comparable, as two different methods of measurement were used.

Similar data are also available for richardii. Bigg (1969b) combined his standard length data from Vancouver Island richardii with those of Bishop (1967, 85) from Tugidak Island, and showed that

the length of fully-grown females averaged 92% of that of males. Thus, it can be seen that, on the basis of standard lengths of fully grown adults, the sexual dimorphism is almost as marked in richardii and largha as it is in stejnegeri.

II. 4. Phoca vitulina richardsi (Gray, 1864)

Halichoerus antarcticus Peale, 1848, p. 30. Single specimen labelled from Kerguelen Islands; later (Gill, 1866) shown to be identical with Phoca of California and Oregon coasts. Nomen oblitum.

Halicyon richardii Gray, 1864, pp. 28-31. Two specimens from British Columbia.

Halicyon richardii gray, 1866, p. 30.

Phoca pealii Gill, 1866, pp. 4, 13. Name substituted for Halichoerus antarcticus.

Phoca richardsi Clark, 1873, pp. 556-557. Single specimen from California. Halicyon richardii referable to Phoca vitulina. Includes emendation by Sclater of Gray's name richardii to richardsi.

Halicyon richardi Gray, 1873, p. 779.

Halicyon richardsi Gray, 1874, pp. 4-5.

Phoca vitulina (part) Allen, 1880, pp. 559-597. Halicyon richardsi listed as a synonym.

Phoca vitulina (part) Nelson and True, 1887, pp. 232, 264-265. Aleutian and Pribilof Islands. Phoca richardsi listed as a synonym.

Phoca (Phoca) vitulina (part) Trouessart, 1897, p. 385. P. richardsi listed as a synonym.

Phoca richardsi Allen, 1902a, p. 225. Alaska Peninsula.

Phoca richardii pribilofensis (part) Allen, 1902b, p. 495. Adak Island and Yakutat.

Phoca richardii geronimensis Allen, 1902b, pp. 495-496. Baja California, Santa Barbara Islands. Large, with heavy dentition.

Phoca (Phoca) vitulina richardsi Trouessart, 1904, p. 287.

Phoca vitulina largha (part) Smirnov, 1927a, pp. 14-15. Phoca richardii included in synonymy.

Phoca vitulina largha (part) Ognev, 1935, pp. 523-541. Pacific Ocean shore of North America as far south as Vancouver Island and St. Hieronymus [=San Geronimo] Island.

Phoca vitulina richardii Schwarz, 1942, p. 222. Pacific coast of North America, south of the Alaska Peninsula, merging in the south with P. v. geronimensis.

Phoca vitulina geronimensis Schwarz, 1942, p. 222. North American coast, southern part. Distinguishable from the northern form (P. v. richardii) by its dark color.

Phoca vitulina richardii (part) Doutt, 1942, pp. 117, 120-121. American side of the North Pacific Ocean.

Phoca vitulina geronimensis Doutt, 1942, pp. 117, 122. American side of the North Pacific Ocean in the vicinity of Lower California.

Phoca vitulina richardi Scheffer, 1958, pp. 92-93. North and west coasts of North America, south to the latitude of Cedros Island; Aleutian Islands.

Phoca vitulina richardi (part) Chapskii, 1960, pp. 355-358. Aleutian Islands, Bering Sea and possibly migrating into Chukchi Sea in summer. Pagophobic.

Phoca vitulina richardi (part) King, 1964, pp. 52, 55, Map 19. Alaskan coast [portion] south to Baja California, Guadalupe Island, Pribilof and Aleutian Islands.

Phoca vitulina richardi (part) Mohr, 1965, pp. 273-287, Fig. 7. Gulf of Alaska, southern coast of Alaska Peninsula, Aleutian Islands, Pribilof Islands.

Phoca vitulina richardi McLaren, 1966, p. 469.

Phoca vitulina richardi (part) Nishiwaki, 1972, pp. 171-172. From southern coasts of the Aleutians south along North American coast to California and Mexico.

Phoca vitulina richardii Burns and Fay, 1973, p. 48. In Bering Sea from southern Bristol Bay to Commander Islands. Those in central and eastern Aleutians similar in many respects to P. insularis (=P. v. stejnegeri).

Early descriptions

Land-breeding harbor seals of the North American coast are currently referred to as P. v. richardii (or richardi). The original description was by Gray (1864) of Halicyon richardii from two specimens collected on the coast of British Columbia. The precise location in which these were collected was discussed by Scheffer and Slipp (1944). A third specimen was examined by Clark (1873) who decided that the three were referable to P. vitulina. Since then richardii has always remained in the genus Phoca,

usually as a subspecies of P. vitulina.

There has been some confusion over the correct spelling of richardii. In the original description Gray dedicated the species "to Captain Richard, the Hydrographer to the Admiralty and Captain of H.M.S. 'Hecate' when these seals were collected". The name was emended to richardsi, "after the Hydrographer to the Admiralty", in an editorial footnote by P. L. Sclater to a paper by Clark (1873). That the Hydrographer's name was in fact Richards is supported by Day (1967) in a review of the British Admiralty Hydrographic Service. Scheffer and Slipp (1944) and King (1964) also stated that the seal was named after Captain G. H. Richards.

The epithet richardsi was used by most nineteenth century authors who wrote about eastern North Pacific harbor seals: Clark (1873); Nelson and True (1887); Allen (1880, 583; 1902a) and Trouessart (1897). Gray (1874) used it too, but also (Gray, 1866; 1873) used richardi. The return to richardii appears to have begun with Allen (1902b, 491), although he gave no reason for changing from his previous usage of richardsi.

The combination P. v. richardsi was first used by Trouessart (1904), and later (as P. v. richardii) by Doutt (1942) and Schwarz (1942). The "ii" ending was commonly used until the late 1950's (also, for example, by Scheffer and Slipp, 1944; Davies, 1958). Then Scheffer (1958) used richardi which has since been in vogue (e.g., King, 1964; Mohr, 1965; McLaren, 1966; Rice and Scheffer, 1968; Burns and Fay, 1970; Nishiwaki, 1972), although there has been a recent return to richardii (Burns et al., 1972; Burns and Fay, 1973).

Gray's subsequent use of richardsi indicates that he had a lapsus

memoriae when he applied the epithet richardii in 1864, and so made an inadvertent error in the sense of Article 32 (a) (ii) (International Commission on Zoological Nomenclature, 1964). The incorrect original spelling should be corrected [Article 32 (c)]. The first emendation of the original spelling is richardsi of Sclater (in Clark, 1873), and hence, applying Article 33 (a) (i), the epithet should be "richardsi (Gray, 1864)".

Attention should also be drawn to the name Halichoerus antarcticus Peale, 1848, that was used for a single specimen originally believed to be from Kerguelen Islands. It was later decided by Gill (1866, in Allen, 1880, 581) that it was identical with harbor seals from the California and Oregon coasts, and had been mislabelled. It was then renamed Phoca pealii. Allen (1880) also examined the specimen and agreed with Gill's interpretation. The name antarcticus was therefore discarded, and so becomes a nomen oblitum.

In a revision of the taxonomy of North Pacific Phocidae, Allen (1902b) retained specific status for richardsi, and described two subspecies: a northern one, P. r. pribilofensis and a southern one, P. r. geronimensis. The former has been discussed on page 30 where it was considered to be largha. The southern form was notable for its large skull and heavy dentition, and will be discussed on page 55.

In a taxonomic review of the Phocidae, Doutt (1942) used the name P. v. richardii for harbor seals of the North American coast, with the exception of those from Baja California, for which he reserved the name P. v. geronimensis. (Doutt's work has been criticized on page 35.)

This system was essentially followed by most later authors (e.g., Davies, 1958; Scheffer, 1958) until Chapskii (1960) invoked an ecological as well as a morphological division of North Pacific harbor seals and pointed out that ice-breeding largha reach the Alaskan coast of Bering Sea. Although Schwarz (1942) had limited the distribution of P. v. richardsi to south of Alaska Peninsula, he was largely ignored.

Distribution and biology

The northern limit of the range of richardsi is still unclear. Populations of harbor seals breeding on land on the northern coast of the Alaska Peninsula and throughout the Aleutian Islands have been reported by Burns (1970) and Mohr (1965), respectively. Harbor seals of the Aleutian and Commander Islands have been included in the range of "insularis" (=stejnegeri) by Belkin et al. (1969), and in the range of richardsi by Burns and Fay (1973) who noted that those of the central and eastern Aleutians showed similarities to stejnegeri. The presence of land-breeding harbor seals in the Pribilof Islands has been discussed on page 32. Hanna (1920) briefly reported on hair seals at St. Matthew Island, without identifying the species. He stated that a colony with many young was sighted on July 8. Land-breeding harbor seals are known to bear their young in late June on the northern coast of the Alaska Peninsula (Burns, 1970), while largha pups are born in late March and early April (Burns et al., 1972). If by "young" Hanna meant small, non-weaned pups, then his report of a colony is suggestive of land-breeding harbor seals. The northern-most known breeding colony of harbor seals on the Alaskan coast is at Egegik, eastern Bristol Bay (J. Vania, voc. comm.).

Land-breeding harbor seals in Bering Sea were subdivided into two groups by Fay (1973) according to their association with sea ice. Those in Bristol Bay have extensive contact with ice during the winter, while those of the Aleutian Islands have little or no contact throughout the year.

Attempts at population estimates of richardsi have been made for British Columbia (Bigg, 1969b; 35,000 animals), Washington (Newby, 1973b; less than 2,000) and Oregon (Pearson and Verts, 1970; 500). In California several aerial surveys of pinnipeds have been made. On the latest (Carlisle and Aplin, 1971), 1,675 harbor seals were counted. At two locations in California, maximum numbers have been observed hauled-out during the late afternoon: in Humboldt Bay (Rosenthal, 1968) and on Ano Nuevo Island (Peterson and Gentry, 1967).

Studies of reproductive biology and life history of richardsi have been made at Tugidak Island by Bishop (1967), in British Columbia by Bigg (1969b) and in Washington by Scheffer and Slipp (1944) and Newby (1973a).

Food habits of richardsi have been studied in Washington by Scheffer and Sperry (1931), in the Gulf of Alaska and southeastern Alaska by Imler and Sarber (1947), and in the Aleutian Islands by Wilke (1957). These authors found that shallow-water fish comprise the main part of the diet. On the other hand, two studies in British Columbia (Fisher, 1952; Spalding, 1964) indicate that salmon are the major prey species at certain times of the year, especially in upriver areas.

The geronimensis phenotype

P. r. geronimensis was first described by Allen (1902b) from five

skulls and a single stuffed specimen from San Geronimo Island, Baja California and Santa Barbara Island, southern California. The skulls were notable for their larger size and heavier dentition compared with "true P. richardii from farther north and with the Pribilof Island skulls" (p. 494). Allen's division was almost immediately subjected to polite criticism by Osgood (1904) who examined all the available material and could not appreciate any characters that would serve to separate the two subspecies. The subspecies was recognized by Doutt (1942) who had seven specimens from San Geronimo Island and two from San Martin Island, Baja California. Although he noted that skulls of old males were very large, robust and heavily ossified with a tendency to form a sagittal crest, he was unable to find any clearly differentiating character between these skulls and others from the eastern Pacific shores. He also commented on the very dark pelage of the six geronimensis skins compared with 23 others from farther north, and decided that these animals could be recognized subspecifically on that basis.

P. v. geronimensis was recognized also by Schwarz (1942) and Scheffer and Slipp (1944) on the basis of its dark pelage. Davies (1958) thought that geronimensis was better differentiated from richardsi than richardsi was from largha, presumably from reports of its heavy skull and dark pelage. He devised a Pleistocene timetable for the evolution of North Pacific phocids in which he reasoned that, because geronimensis is the most differentiated of these harbor seals, it was the first to arrive.

In a description of mammals of Baja California, Huey (1964) believed that Doutt's P. v. geronimensis was a valid subspecies, although he had

not examined any specimens. He stated that its range extended north to the California boundary and included San Ignacio Lagoon, San Geronimo and San Martin Islands. Other than this, the geographical separation between richardsi and geronimensis has not been mentioned. Scheffer and Slipp (1944) suggested that they intermingled. Thus there does not seem to be any barrier to prevent gene-interchange between these two taxa. Also, one of the characters which has been used to distinguish geronimensis (dark pelage) is known to occur in Gulf of Alaska richardsi (Bishop, 1967, 99). Consequently, it seems reasonable to consider geronimensis as synonymous with richardsi until a detailed comparison of the two indicates otherwise.

It is interesting to note that both geronimensis and stejnegeri are distinguished by their heavy skull and dark pelage. Although Allen (1902b) first described both geronimensis and stejnegeri and noted the large skull of each, he evidently did not compare them directly. A glance at his data (Allen, 1902b, 498) shows that his P. stejnegeri skulls are not very different from those of P. r. geronimensis.

Comparison of stejnegeri and richardsi

In this survey of the literature relating to richardsi and stejnegeri, the paucity of characters which might serve to distinguish them is apparent. This problem is particularly acute in those areas where their ranges are thought to overlap. Dark pelage and heavy skull have been used to characterize stejnegeri, but these features are not unique to it. Nor does there appear to be any barrier to prevent genetic exchange between them. Indeed, Chapskii (1969), after examining five harbor seal skulls

from North America and comparing them with those described by Gray (1864) and Allen (1902b), considered that all of the pagophobic harbor seals of the North Pacific should be considered as a single species. Thus, richardsi and stejnegeri should only be considered subspecifically distinct at this stage. Consequently, any character that can be shown to differ significantly between specimens from, say, Hokkaido or the southern Kurils on the one hand, and those from California or Washington on the other, should also be examined in specimens from several intermediate locations before it can be displayed as demonstrating diagnostic value. Two recently reported characters for stejnegeri (Belkin et al., 1969; Naito and Nishiwaki, 1972b; 1973) that seem worthy of further investigation are its hyoid (much reduced compared with largha) and its grey lanugo in foetal and some post-partum pups (contrasting with the white lanugo in largha and most richardsi).

III. LITERATURE REVIEW: GENETIC VARIATION DETECTED BY ELECTROPHORESIS

III. 1. Estimates of Genetic Variation in Populations

After the development of gel electrophoresis by Smithies (1955), and the demonstration by Hunter and Markert (1957) that enzymes could be detected on gels by histochemical staining techniques, attention was directed towards describing polymorphisms in species of domestic animals and man [reviewed by Lush (1966) and Giblett (1969)]. In recent years, there have been many estimates of the amount of electrophoretically detected genetic variation in naturally-occurring populations. Usually about 20 enzymes chosen at random (i.e., with no previous knowledge regarding the likelihood of their being polymorphic) are examined in one or more populations. Such studies provide useful points of reference with which investigations on Alaskan marine mammals can be compared.

The amount of genetic variation is usually measured by one or more of three statistics. Most commonly used is the proportion of polymorphic loci per population, denoted by P. In some early reports, the proportion of polymorphic proteins was estimated, but as the structure of some proteins is controlled by more than one locus, this statistic should be considered on a locus basis. The number of loci which are polymorphic in at least one population is sometimes used to obtain an estimate of the proportion of polymorphic loci over the whole species. Unfortunately, this is often confused with P.

The other statistics in use are the mean number of alleles segregating per locus per population, and the proportion of heterozygous

loci per individual (H). To obtain the latter, the proportions of heterozygotes at each locus (h) are summed and divided by the number of loci examined. For this calculation, either the observed proportion of heterozygotes, or the expected proportion (based on calculations made from observed frequencies and the Hardy-Weinberg equilibrium) is used.

A problem associated with estimating the first two statistics is that more than one criterion is used for the functional definition of polymorphism. In their extensive studies of genetical variation in human populations, Harris and his colleagues (e.g., Harris and Hopkinson, 1972) have considered a locus to be polymorphic if the frequency of the most common allele is not greater than 0.99. This is frequently referred to as the 0.99 (or 0.01) criterion for polymorphism. On the other hand, Selander and colleagues in their studies on genetic variation in vertebrate populations, frequently use the 0.95 criterion for polymorphism (e.g., Avise and Selander, 1972), although in several cases they have neglected to state their criterion.

The original definition of polymorphism (Ford, 1955) required the rarer form to be present with a frequency greater than could be maintained by recurrent mutation. The working definitions will include fewer polymorphisms than would Ford's definition. The more rigorous criteria are dictated by sample size; the probability of detecting a polymorphic system with a gene frequency of, say, 10^{-5} when the sample size is 10^{2}, is very small (viz. 0.001).

This problem of defining polymorphisms does not arise when the amount of genetic variation is measured by the proportion of loci

heterozygous per individual. For this reason H is the preferred statistic for comparing the amount of genetic variation between species, although P has been used more commonly. A method for estimating the standard deviation of H, based on the binomial distribution of h values has been provided by Powell (1971), but has scarcely been used.

Estimates of these parameters are provided in tabular form for several species by Gottlieb (1971). Some other estimates for vertebrate populations are provided in Table 3. It can readily be seen that the amount of genetic variation varies considerably between species. For instance, P varies from zero in Mirounga angustirostris to 0.56 in Coturnix coturnix, and higher in some Drosophila species [e.g., 0.71 in D. equinoxialis (Ayala, Powell and Tracey, 1972)]. Two higher values for Drosophila that are occasionally seen in the literature are misleading (e.g., in the review by Gottlieb, 1971). One of these is the value for P of 0.73 for D. melanogaster (Berger, 1970). As five of the six loci examined in that study were already known to be polymorphic, that estimate is biased. The value of 0.83 in the D. willistoni group of species (Ayala et al., 1970) is not an estimate of P, as it represents the proportion of polymorphic loci in the species. Unfortunately, Gottlieb (1971) fails to make the distinction between the proportion of polymorphic loci per population and the proportion per species. Most values are on a population basis, but at least three (that for D. willistoni referred to above, and those for Mus musculus) pertain to the whole species.

These measures of the incidence of genetic variation in populations are concerned solely with variation which results in electrophoretically

TABLE 3. Estimates of genetic variation in vertebrate populations.

Species	Reference	Number of populations	Number of loci	Proportion of polymorphic loci per population	Proportion of heterozygous loci per individual
Homo sapiens	Harris and Hopkinson (1972)	1	71	.28	.067
Dipodomys sp.	Johnson and Selander (1971)	11 species	17	0 to .22	0 to .051
Peromyscus floridanus	Smith, Selander and Johnson (1973)	3	39	.21	.053
Sigmodon hispidus	Johnson et al. (1972)	5	23	.082	.021
S. arizonae		1	23	.091	.029
Thomomys bottae	Patton, Selander and Smith	5	27	.23	.071
T. umbrinus	(1972)	1	27	.11	.033
Spalax sp.	Nevo and Shaw (1972)	4 species	17	-	.018 to .056
Mirounga angustirostris	Bonnell (in litt., 1972)	5	20	0	0
Zonotrichia capensis	Nottebohm and Selander (1972)	4	16	-	.035
Coturnix coturnix	Baker and Manwell (1967)	2	24	.56	-
Astyanax mexicanus	Avise and Selander (1972)				
Cave dwelling		3	17	.14	.036
Surface dwelling		6	17	.37	.11
Sebastes alutas	Johnson, Utter and Hodgins	3	25	.08	.038
S. caurinus	(1973)	1	25	.04	.018
S. elongatus		2	24	.08	.032
Anolis sp.	Webster, Selander and Yang (1972)	4 species	29	0 to .15	0 to .053

detectable differences, i.e., changes in proteins involving the charged amino acids lysine, arginine, aspartic acid and glutamic acid, that change their net charge. Harris (1970, 16) predicted that about one-third of all possible mutations which result in amino acid substitutions will cause a change in net charge of the resulting protein. Another estimate, obtained from the matrix of observed amino acid substitutions provided by Dayhoff, Eck and Park (1972, Fig. 9-7), is 0.29. Thus the data summarized in Table 3, and elsewhere, clearly underestimate the total amount of genetic variation in soluble enzymes actually present in populations.

Certain classes of enzymes are thought to be more susceptible to polymorphic variation than others. Johnson (1971) proposed that, if polymorphisms are maintained by natural selection, those enzymes in pathways that control irreversible and rate-limiting steps should be most sensitive to selection and most likely to be polymorphic. Consequently, he predicted that the regression of a measure of the amount of variation at a locus on the equilibrium constant of the reaction should be positive, and demonstrated this with data from 14 enzymes in Drosophila sp. However, Ayala and Powell (1972) examined 25 to 28 loci in three sibling species of the D. willistoni group, and were unable to support Johnson's hypothesis. They further pointed out that Johnson used some equilibrium constants which do not obtain at physiological pH.

Several authors have shown that enzymes active in energy metabolism are less variable than other enzymes. Gillespie and Kojima (1968) working

with D. ananassae found mean values of H of 0.026 and 0.190 for these two groups of enzymes, based on seven and four enzymes respectively. The same tendency has been noted in other species of Drosophila by Kojima, Gillespie and Tobari (1970), Ayala and Powell (1972) and Richmond (1972); in Mus musculus by Selander and Yang (1969); and in humans and the monkey, Macaca nemestrina by Cohen et al. (1973). The last authors suggested that enzymes whose main metabolites cannot readily be supplied by another pathway (such as the glycolytic enzymes) would be expected to show less variation than enzymes whose metabolites come from several pathways. Selander and Yang (1969) extended their comparison of the amount of genetic variation among groups of proteins in M. musculus with different functional characteristics to include non-enzymatic proteins encoded by ten loci. The amount of variation controlled by these loci ($P = 0.20$) did not differ significantly from that of loci controlling enzymes involved in energy metabolism ($P = 0.28$). Thus, when estimates of the amount of genetic variation in different populations are compared, or their significance is considered, the function of the enzymes examined should be taken into account.

III. 2. Comparisons of Populations Using Electrophoretic Data

In order to distinguish groups of similar populations from a set of populations, comparisons have frequently been made on the basis of allele frequency data obtained from electrophoretic studies of one, or at most, a few, proteins, e.g., Kirsch and Poole (1967) in the grey kangaroo, Macropus giganteus; de Ligny (1971) in fish; and Nadler et

al. (1973) in ground squirrels, Spermophilus sp. Such studies have also been made in pinnipeds: they are reviewed in the following section. Genetic similarities of populations are more reliably assessed when data are accumulated for several proteins, but then a locus-by-locus comparison becomes cumbersome, and it is necessary to summarize the data for all loci with a single statistic.

The simpler and less efficient statistics that have been suggested are based on the proportion of proteins or of alleles common to the two populations being compared. The proportion of proteins was used by Hubby and Throckmorton (1965; 1968) in studies of Drosophila populations. Selander, Hunt and Yang (1969) compared their data on Danish house mouse populations M. musculus musculus and M. musculus domesticus) with those of Hubby and Throckmorton for Drosophila by considering the proportion of loci which share identical alleles. Ayala et al. (1970) used a numerical index of genetic dissimilarity to compare two species. This was based on the alleles present at each locus examined, and took values from zero to five.

There are several coefficients of genetic similarity of pairs of populations which make use of allele frequency data, e.g., those of Sneath (in Sokal and Sneath, 1963), Stewart (in Rogers, 1972), Cavalli-Sforza and Edwards (1967), Kidd and Pirchner (1971), Nei (1972), Nevo and Shaw (1972) and Rogers (1972). Another coefficient of genetic similarity is based on genotype frequency data (Hedrick, 1971). Five of these parameters have been reviewed by Rogers (1972) who applied them to a set of allele frequency data for 41 loci from six populations

of Danish house mouse taken from Selander et al. (1969). These coefficients essentially gave the same result, in that the populations fell into two distinct groups, according to subspecies, but there were some differences in the ordering of populations within subspecies.

Rogers (1972) also reviewed some approaches to the measurement of genetic distance, again using allele frequency data, which are based on Mahalanobis' (1936) concept of generalized distance. He pointed out that these statistics are dependent upon sample size. All other statistics available for population comparisons appear to ignore this important consideration.

Rogers' coefficient of genetic similaritiy, S, can take values from one (when the two populations being compared are identical for each locus examined), to zero (when the populations do not share any alleles). It can be converted into a distance coefficient simply by subtracting it from unity. One of its desirable features is that equal differences in allele frequency in the two populations being compared produce the same contribution. This feature is absent from most other such statistics.

Rogers' S has been used by Selander and colleagues for population comparisons within several vertebrate taxa. Values of the coefficient have been tabulated by Avise and Selander (1972) for the following groups: the fish, Astyanax mexicanus; house mouse, M. musculus; kangaroo rats, Dipodomys sp.; cotton rats, Sigmodon sp.; the field mice, Peromyscus polionotus and P. floridanus; Anolis lizards; and side-blotched lizards, Uta stansburiana. Another comparison, for pocket gophers, Thomomys sp., was reported by Patton, Selander and Smith (1972). In these studies,

the mean value of S ranges from 0.75 to 1.00 for conspecific comparisons, and from 0.21 to 0.77 for interspecific comparisons. In each study, values of S were greater for conspecific than interspecific comparisons. These data provide points of reference for similar studies of harbor seal populations.

III. 3. Biochemical Polymorphisms among Pinnipeds

Among pinnipeds, allelic variation demonstrated by electrophoresis has been used in comparisons of populations in a few species, but not in the elaborate manner described above, as only small numbers of proteins have been examined. Populations of the harp seal, Pagophilus groenlandicus, from eastern and western North Atlantic were separated statistically on the basis of transferrin gene frequencies by Naevdal (1966a). This separation agreed with that determined earlier on the basis of cranial measurements by Yablokov and Sergeant (1963), who proposed that the populations were reproductively isolated. However, a later series of blood samples from the western population cast some doubt on this interpretation (Naevdal, 1969). An attempt by Borisov (1966) to distinguish immunologically between small numbers of harp seals from different populations was also unsuccessful.

Populations of southern fur seals, Arctocephalus forsteri and A. pusillus in Australia and New Zealand, could be distinguished by variations in both transferrin and a haem-binding protein (Shaughnessy, 1970). This division is consistent with that based on cranial characters proposed by King (1969) and later supported by Repenning, Peterson and Hubbs (1971).

Blood proteins have been examined electrophoretically in one other group of Arctocephalus consisting of six animals (Seal, Erickson, Siniff and Hofman, 1971). No electrophoretic variation was reported. However, the authors did not indicate which of the eight proteins mentioned in their paper were examined in Arctocephalus. They listed this species as A. tropicalis without stating the collecting locality. As Erickson et al. (1970) reported collecting blood from six fur seals during a cruise in the South Orkney and South Shetland Islands, it would seem that the fur seals examined by Seal, Erickson, Siniff and Hofman (1971) were A. gazella rather than A. tropicalis.

Populations of the Weddell seal, Leptonychotes weddelli, from four widely spaced localities in Antarctica were distinguished by transferrin gene frequencies by Shaughnessy (1969). As Weddell seals breed in the vicinity of their birth place (Stirling, 1969), local populations which differ genetically are not unexpected. Transferrin polymorphism in Weddell seals has also been examined by Seal, Erickson, Siniff and Hofman (1971) who assumed that they observed two of the three phenotypes observed by Shaughnessy (1969). They noted a difference in allele frequency between their Ross Sea and Weddell Sea series which they described as "barely significant". Their χ^2 value (2.83) must be based on numbers of genotypes. It has a corresponding probability of .093, which is significant only at the 10% significance level. Nor is the χ^2 value based on the number of alleles significant ($\chi^2 = 2.38$, $P = 0.13$). Thus it should be concluded that no significant difference was found in transferrin frequency between their Ross Sea and Weddell Sea series.

The amount of genetic variation within populations has been investigated in both species of Mirounga. In M. leonina of Macquarie Island, Shaughnessy (in prep.) examined five blood proteins and three milk proteins controlled by a total of 12 loci, and found that 0.17 of these loci were polymorphic, and that the proportion of heterozygous loci per individual was 0.075. The two polymorphic loci controlled milk proteins. The possibility that this variation might be a function of the stage of lactation and not genotype was considered.

Another investigation of this population was performed by McDermid, Ananthakrishnan and Agar (1972) who examined 17 blood proteins in 42 animals, and found variation in five of them. (These authors reported that they had examined 19 proteins. On close inspection of their data, however, it is apparent that four of the proteins either produced poor patterns or were absent, and so could not be examined for variation, and that two others seem to have been omitted from the count, probably malate dehydrogenase and the second system of haem-binding proteins, neither of which showed variation. Hence, it can be concluded that they observed variation in five of 17 proteins.) At least three of these 17 proteins are controlled by two loci (LDH, haptoglobin and haemoglobin). Thus, of 20 loci examined for variation in the Macquarie Island population of M. leonina by McDermid et al. (1972), the proportion found to be polymorphic is 0.25. Combining information from the two studies on this population, we find that of a total of 23 different structural loci (nine of which were examined in both studies), seven, or a proportion of 0.30, are polymorphic. Unfortunately the data of McDermid et al. (1972) cannot

be used to estimate the proportion of heterozygous loci per individual in this population. They suspected that their series was from related individuals, but having neglected to determine family relationships, could not calculate gene frequencies.

Another study relevant to an estimation of the amount of genetic variation in M. leonina is that of Seal and his co-workers (Seal, Erickson, Siniff and Cline, 1971; Seal, Erickson, Siniff and Hofman, 1971). No variation was reported in the proteins haemoglobin, transferrin, LDH, haptoglobin, albumin, malate dehydrogenase, and thyroxine-binding proteins, although each of the five animals in the former study was observed to have a single transferrin zone, while each of the 18 animals in the second study possessed a double zone. This may have been due to changes in technique rather than genetical variation between animals, as McDermid et al. (1972) reported a single zone of transferrin in Macquarie Island animals, while two zones were observed by Shaughnessy (in prep.). The source of Seal's animals was not mentioned, but presumably they were from fringe areas of the stock that is centered on South Georgia (Siniff and Cline, 1968; Erickson et al., 1970).

After observing only two LDH zones in M. leonina serum and at least four LDH zones in sera from Hydrurga leptonyx, L. weddelli, Lobodon carcinophagus and Ommatophoca rossi (which they referred to the subfamily Lobodontinae), Seal, Erickson, Siniff and Hofman (1971) suggested that Phocidae might be separable at the subfamily level on the basis of LDH pattern. King's (1966) abandonment of the subfamily Cystophorinae, and placement of Mirounga in the Monachinae together with the four antarctic

species (also followed by Burns and Fay, 1970), means that such a separation would be at a lower level. However, observations of up to five serum LDH zones in M. leonina by McDermid et al. (1972) refute this possibility of distinguishing between M. leonina and the antarctic species.

In M. angustirostris, Bonnell (in litt., 1972) examined 18 blood proteins encoded by 20 loci in 84 to 124 animals from 5 populations without observing any variation. He suggested that this homogeneity is the result of random gene fluctuations leading to fixation during the near extermination of this species during the nineteenth century (Bartholomew and Hubbs, 1960).

In some other pinnipeds, several proteins have been examined electrophoretically, but no efforts have been made to quantify the amount of variation. Naevdal (1971) reviewed his investigations of the proteins haemoglobin, haptoglobin and transferrin in four North Atlantic phocids. He found variation in transferrin of harp seals (referred to above), in a protein thought to be transferrin in ringed seals, Pusa hispida, and in haptoglobin of hooded seals, Cystophora cristata. In his report of the haptoglobin variation (Naevdal, 1966b), it appears that bands I and II in Figure 5 are reversed. Although individual differences in haptoglobin are illustrated in this figure, the described polymorphism is better illustrated in the preceding figure. No variation was observed in bearded seals, Erignathus barbatus. Naevdal also examined pancreatic amylase in hooded seals (which were polymorphic) and harp seals (monomorphic). Seal, Erickson, Siniff and Hofman (1971) examined eight

proteins (haemoglobin, transferrin, LDH, haptoglobin, albumin, malate dehydrogenase, thyroxine-binding globulin and thyroxine-binding prealbumin) in the four antarctic phocids. They found variation in transferrin of Weddell seals (mentioned above), and in both transferrin and LDH of crabeater seals, L. carcinophagus. Blumberg, Allison and Garry (1960) described variation in haptoglobins and postalbumins of northern fur seals, Callorhinus ursinus. The haptoglobin variation is most likely an artifact, and will be discussed on page 106. Bonner and Fogden (1971) observed variation in a serum protein in harbor seals and in grey seals, Halichoerus grypus, from Britain. As the identity of these proteins was not determined, and the differences appear to concern minor zones, their use in population identification would be unreliable.

Little work has been done on blood groups in pinnipeds. Fujino and Cushing (1960) described four types of antigenic specificities in C. ursinus. Their non-random distribution amoung pelagic animals during the migratory season suggested the presence of population differences. Cushing (1964) made brief reference to variation in red cell antigens in five Zalophus californianus. Serological studies on some seals of the sub-genus Pusa were performed by Taliev (1940, in Brooks, 1950). He found that the Baikal seal (P. sibirica) showed closer affinity to the ringed seal of arctic seas (P. hispida hispida) than to the Lake Ladoga seal (P. hispida ladogensis) or the Caspian seal (P. caspica). McLaren (1960) did not consider that result to be significant of relationships. Since the Ladoga seal is but a recently isolated and little differentiated ringed seal, McLaren expected it to show the closest affinity to the

arctic ringed seal. However, it would seem that McLaren was criticizing Taliev for a comparison that the latter did not even consider. Taliev's serological observations can be expressed phylogenetically in a cladogram (Fig. 4, after Sarich, 1969a). In the absence of more information on serological relationships among Pusa, the cladogram indicates that Taliev's observations and McLaren's concepts of Pusa evolution do not contradict each other.

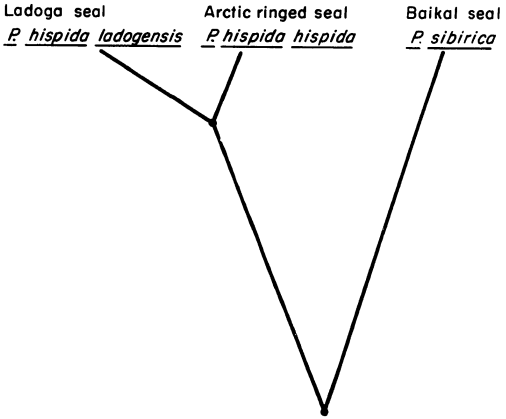

Figure 4. A possible phylogeny (cladogram) of three taxa of the sub-genus Pusa, based on the serological observations of Taliev (1940) and the evolutionary concepts outlined by McLaren (1960).

IV. MATERIALS AND METHODS

IV. 1. Collection of Blood Samples

Blood-collecting techniques

Blood samples from live harbor seals and other Bering Sea pinnipeds (ribbon seal, Histriophoca fasciata; bearded seal, Erignathus barbatus; walrus, Odobenus rosmarus; and Steller sea lion, Eumetopias jubata) were taken by lumbar puncture of the intravertebral venous sinus. A needle was introduced between adjacent dorsal spines of the vertebral column in the lumbar region. Once the ligamentum flavum was punctured, blood was aspirated with a syringe. Insertion of the needle and location of the sinus were facilitated by placing the seal in a flexed position. This sinus was described from harbor seals and grey seals, H. grypus, by Harrison and Tomlinson (1956); in an otariid, Z. californianus, they found only the typical mammalian paired veins ventral to the vertebral column. The ease with which blood was drawn from the walrus and Steller sea lion in this study indicates that these species have an intravertebral sinus in a position similar to that in harbor seals.

In some other studies in which blood was collected from pinnipeds, it was taken from a vein in the hind flipper, e.g., by Chuba et al. (1970) in P. vitulina, Z. californianus, and C. ursinus, and by Naevdal (1969) and Suderman, Yeh-Ku and Ronald (1973) in P. groenlandicus. Blood has also been collected from live pinnipeds by jugular or carotid puncture in Z. californianus by Palumbo et al. (1971), and by cardiac puncture in Arctocephalus sp. by Shaughnessy (1970). Since the lumbar

puncture is simpler and less traumatic, these other techniques were not attempted in this study.

Needles used were 1-1/2" x 18g for young pups, 2" x 18g for large pups and subadults, and 3" x 18g for adults. Animals were forcibly restrained by assistants: at least one assistant for pups, and at least two for older animals.

Blood was drawn into dry bottles and allowed to clot. After centrifugation, or standing overnight if electricity was not available, the serum was removed with a disposable pipet. Red blood cells were then removed from the clots by gently shaking with 0.9% saline, washed twice with saline and haemolyzed by freezing. In the field, sera and haemolysates were frozen, and kept frozen, using a Linde LD 19 liquid nitrogen container (Union Carbide Corporation, Speedway, Indiana). In the laboratory they were stored at -10°C.

Source of blood samples

Series of blood samples from coastal harbor seals (P. v. richardsi and P. v. concolor) were collected at eight localities (Fig. 5). Most were collected from individuals in breeding colonies, or that had been captured from breeding colonies; the others were collected outside the breeding season (Table 4). The California-Washington series originated from southern Humboldt Bay (41° N, 124° W; 19 animals), Westport (47° N, 124° W; 3 animals), Tomales Bay (38° N, 123° W; 1 animal) and Big Sur (36° N, 122° W; 1 animal). The North Atlantic series originated from Boothbay Harbor (44° N, 70° W; 1 animal), Bar Harbor (44° N, 68° W; 3 animals), Sable Island (44° N, 60° W; 12 animals) and Halifax (45° N, 64° W; 1 animal).

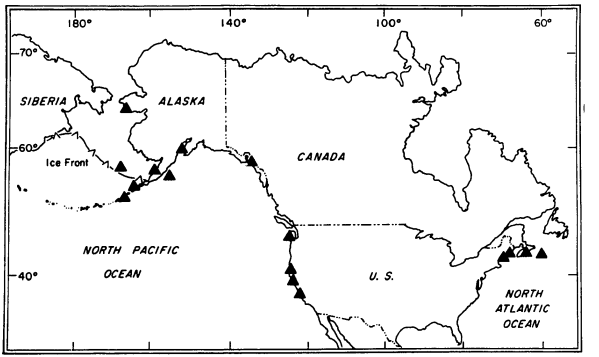

Figure 5. Sampling localities of Phoca vitulina. The ice front represents the approximate southern limit of the Bering Sea ice pack in March-April.

TABLE 4. Number of serum and red cell haemolysate samples collected from Phoca vitulina.

Locality	Date of collection	Months from breeding season	Age group	From breeding localities: in breeding season	From breeding localities: not in breeding season	From captive animals	Total
P. v. richardsi							
Tugidak Island	June, 1970	--	Pup	47	--	--	47
Port Heiden	July, 1970	--	Pup	48	--	--	48
Izembek Lagoon	June, 1970 to August, 1972	--	Pup	3	--	6	
			Subadult	1	--	--	
			Adult	1	--	--	11+
Cook Inlet	July, 1972	--	Pup	--	--	1	1*
Unalaska Island	April, 1972	2 before	Subadult	--	3	--	
			Adult	--	10	--	13*
Juneau	November, 1972	5 after	Pup	--	1	--	
			Subadult	--	1	--	
			Adult	--	6	--	8*
California-Washington	September, 1971	5 after	Pup	--	2	14	
			Subadult	--	1	4	
			Adult	--	0	3	24+

TABLE 4. continued

Locality	Date of collection
P. v. concolor	
North Atlantic	August, 1972
P. v. largha	
Feather Lagoon	September, 1970
"Front" Zone of Bering Sea ice pack	April, 1971 and April, 1972

*No red cell haemolysate samples.
**Includes six mother-pup pairs.
+Includes one mother-pup pair.

Months from breeding season	Age group	From breeding localities: in breeding season	From breeding localities: not in breeding season	From captive animals	Total
5 after	Pup	--	--	15	
	Subadult	--	--	2	17
5 after	Pup	--	3	--	3
--	Pup	58	--	--	
	Adult	8	--	--	66**

The large series from P. v. largha was obtained from the front zone of the Bering Sea ice pack, to the north and northeast of the Pribilof Islands. The three specimens from Feather Lagoon (65° N, 166° W) have been identified as P. v. largha (J. J. Burns, voc. comm.); this locality is beyond the known breeding range of richardsi.

Pups with a fresh, bloody umbilical cord were considered to be newborn, i.e., born within the previous day or two (J. J. Burns, voc. comm.). A total of 14 such newborn pups are included in the material; 12 in the largha series and two in the Izembek Lagoon richardsi series. Also included in the latter series is a full-term foetus.

Pups in the Bering Sea series were all unweaned, and so must have been less than four weeks of age, as Burns and Fay (1973) have indicated that they are suckled for about four weeks. Most pups at Tugidak Island and Port Heiden are born during June (J. S. Vania, voc. comm.). The series from the former locality was taken three weeks before that from the latter. Although the absolute age of these pups was not determined, those from Port Heiden were, on the average, three weeks older than those from Tugidak.

Collections were also made from other Bering Sea pinnipeds. Material was obtained from a mother-pup pair and an adult male of the bearded seal. The pup was estimated to be four to six days old at the time of blood collection, the last three of those days having been spent in captivity. Material was collected from four ribbon seals, comprising one subadult and three unweaned pups, one of which was newborn. Blood was also collected from an adult female Steller sea lion, and an adult

female walrus. The bearded seal mother and pup were caught in the front zone about 100 miles southwest of St. Matthew Island. The ribbon seals and Steller sea lion were collected in the same area as the P. v. largha series. The walrus and adult male bearded seal were caught in the vicinity of St. Matthew Island.

IV. 2. Gel Electrophoresis

Electrophoresis was performed in both starch and acrylamide gels. For vertical acrylamide gel electrophoresis, a water-cooled apparatus (E. C. Apparatus Corporation, Pittsburg, Pa.) was used. The gel slab measured 17.6 x 13.8 cm x 3 mm which required 180 ml of gel solution. Gels were made with 7% acrylamide (70 gm Cyanogum 41, or 66.5 gm acrylamide and 3.5 gm N,N'methylenebisacrylamide per litre), and polymerized with 1.0 ml N,N,N',N'-tetramethylethylenediamine (TMED) as a catalyst and 1.0 gm ammonium persulphate as activator. The addition of the TMED to gel buffer solutions raised their pH by 0.4 units. Catalyst residues were removed from the gel by running for ten minutes before the samples were added. Pre-cast slots for sample insertion were formed with a twelve-place pocket-forming tool of polyvinyl chloride. Samples were introduced into these slots with a 10 µl micro-syringe. Samples (either serum or red cell haemolysate) were diluted with 40% sucrose to make them heavy enough to displace electrode buffer from the slots. A small amount of bromophenol blue (.005 g/ml) was added to the samples to make them more visible during loading. This dye migrated as a blue line with the "front" of discontinuity during electrophoresis with discontinuous buffer systems

and so provided an indication of the progress of electrophoresis. A buffer pump circulated electrode buffer between the two electrode compartments during electrophoresis in order to keep the composition of the solutions identical.

Horizontal starch gel electrophoresis was performed in another water-cooled apparatus (E. C. Apparatus Corporation). The gel slab measured 29.5 x 23.0 cm. Gels were prepared by heating starch with buffer solution in a flask over an open flame until it gelled, and then for another 15 seconds. The flask was shaken vigorously during this process. The gel was then degassed by boiling it under reduced pressure for 15 seconds using a vacuum pump. Hydrolysed potato starch (Electrostarch Co., Madison, Wisconsin) was used in a concentration of 13%. Six hundred ml of hot liquid starch was poured into the gel tray to form a layer approximately 6 mm thick. This was allowed to cool for ten minutes before samples were applied on 8 x 4 mm pieces of Whatman No. 1 filter paper which were pushed into the gel. Sample insertion was about 10 cm from the cathodal end and consisted of about 20 µl of serum or haemolysate, diluted as required for the staining reaction. Electrophoresis was begun about 20 minutes after the gel was poured, and the pieces of filter paper were removed 30 minutes later. After electrophoresis, starch gels were sliced with a fine stainless steel wire on a gel slicing device (Buchler Instruments, Inc., Fort Lee, N. J.) before being stained.

IV. 3. Electrophoretic Buffer Systems and Staining Procedures

Procedures used to examine seven proteins in harbor seals are

described, together with procedures for another two proteins that stained in blood samples from humans but not from harbor seals, and a third protein that stained too weakly to be typed routinely.

Esterase

Esterases were examined by starch gel electrophoresis using the continuous lithium citrate buffer system at pH 5.3 described by Scott and Weaver (1970). Twenty µl samples of undiluted serum were electrophoresed for 5 to 6 hours at 350 volts, at which stage albumin had migrated approximately 12 cm from the origin. Esterase zones were visualized with a stain containing α-napthyl acetate as substrate, and the dye fast blue RR (diazotized 4'-amino-2',5'-dimethoxy-benzanilide) at pH 7.4 (Scott and Weaver, 1970). The release of α-napthol from the acetate, followed by coupling of napthol with the diazo salt, caused the appearance of blue zones at sites of esterase activity after incubation at 37°C for 30 minutes.

Haemoglobin

Haemoglobins were examined by acrylamide gel electrophoresis using the continuous buffer system of Boyer, Fainer and Naughton (1963) which consisted of tris (hydroxy methyl) amino methane, boric acid and EDTA at pH 8.5. Three µl samples of red cell haemolysate diluted 1:40 with sucrose solution were electrophoresed for 2 hours at 300 volts. Gels were stained with amido black solution (3.7 gm amido black, 250 ml water, 250 ml methanol, 50 ml acetic acid) for 30 seconds. Excess stain was removed and zones fixed by washing the gel in a 5:5:1 mixture of water, methanol and acetic acid. Electrophoresis of haemoglobin was also

performed in the same manner at pH 7.8 and pH 9.3.

Haptoglobin and haemopexin

The haem-binding proteins haptoglobin and haemopexin were examined by gel electrophoresis in both starch and acrylamide using a discontinuous buffer system in which the gel buffer was Tris-citric acid at pH 7.4 (Kristjansson, 1963), and the electrolyte was lithium hydroxide-boric acid at pH 7.5. Since poor resolution of the haem-binding zones was obtained on acrylamide gel, the methods outlined will relate to the technique with starch.

Samples consisted of serum with added red cell haemolysate from the same species. The haemolysate was diluted 1:1 with water and then added to the serum in a ratio of 1:7. This amount of haemolysate provided sufficient haemoglobin to saturate the haptoglobin without providing so great an excess as to blur the gels. Gels were electrophoresed at 700 volts for 3 hours, by which time the front of discontinuity had migrated about 7.5 cm from the origin. Haem-binding proteins have peroxidase activity, and so can be located by staining with o-dianisidine (3,3'-dimethoxybenzidine) dissolved in a minimum amount of hydrochloric acid, and then buffered at pH 4.7 in the presence of hydrogen peroxide (Owen and Smith, 1961). After incubation at 37°C for 10 minutes, dark brown zones of oxidized dianisidine indicated the presence of peroxidase activity. This staining mixture was also tried with o-dianisidine dihydrochloride, but without success. Stained gels were washed in 50% methanol for two minutes which makes them white and opaque to provide a good background for the brown zones. Gels stained in this manner could

be stored for a few days in water without becoming brittle. Those of importance were photographed immediately.

Lactate dehydrogenase

Lactate dehydrogenase was examined by acrylamide gel electrophoresis using the continuous citric acid-dibasic sodium phosphate buffer system of Fine and Costello (1963) at pH 7.0. Three µl samples of serum diluted with an equal volume of sucrose solution were electrophoresed for 4 hours at 200 volts. Higher voltages caused overheating and strange patterns resulted. After electrophoresis, LDH zones were visualized with a stain consisting of L-lactic acid buffered at pH 8.5, nicotine-adenine nucleotide (NAD, a co-factor), phenazine methosulphate (PMS) and nitro blue tetrazolium (NBT) as described by Fine and Costello (1963). Electrons from the oxidation of lactate to pyruvate are transferred from NADH to NBT which is reduced to formazan in the presence of PMS. Sites of LDH activity were indicated by the blue formazan zones after incubation at 37°C for 30 minutes. The reaction worked equally well with NBT replaced by tetra nitro blue tetrazolium, in which case LDH activity was indicated by brown zones. Since this stain is light sensitive, it was necessary to incubate and store the gels in darkness.

Peptidase

Peptidase was examined by starch gel electrophoresis using the continuous Tris maleate buffer system of Lewis and Harris (1967) at pH 7.4. Twenty µl samples of red cell haemolysate diluted with an equal volume of water were electrophoresed for 3 hours at 300 volts. Peptidase zones were visualized with a stain containing the peptide leucyl-glycyl-glycine

as substrate, crude Crotalus adamanteus venom (Sigma) as the source of L-amino acid oxidase, peroxidase POD II (Boehringer), manganese chloride and o-dianisidine hydrochloride. These components were applied in an agar overlay. The composition of the stain mixture and the reaction sequence are described by Lewis and Harris (1967). After incubation at 37°C for 30 minutes, dark brown zones of oxidized dianisidine indicated active peptidase sites.

Transferrin

Transferrin was examined by acrylamide gel electrophoresis using a discontinuous buffer system based on that of Kristjansson and Hickman (1965). The gel buffer consisted of 0.012M cacodylic acid (hydroxydimethyl arsine oxide) and 0.009M Tris. At the concentration recommended by these authors, the gel wrinkled and shrank in the vicinity of the slots. This undesirable effect was eliminated by reducing the concentration of cacodylic acid by a quarter. The electrolyte comprised 0.191M boric acid and 0.019M lithium hydroxide at pH 7.5. Three µl samples of serum diluted 1:4 with sucrose solution were electrophoresed for 2 hours at 300 volts. After electrophoresis, gels were stained, washed and fixed in the same manner as that described for electrophoresis of haemoglobin.

Transferrin zones were identified by autoradiography using Fe^{59} in the form of ferric chloride (New England Nucleides). One drop (2 µcuries) of a solution of suitable activity was evaporated to dryness at 40°C in a 10 x 75 mm culture tube. One drop of serum and one drop of sucrose solution were added to the tube and allowed to stand for one hour at room temperature. Five µl of this mixture (containing 0.2 µc of activity)

was applied to the gel. After electrophoresis the gel was sliced, and one half was stained with amido black. The other half was enclosed in a single layer of "Saran Wrap", frozen, and applied to a 10 x 8" Kodak No Screen Medical X-ray Film in an empty 10 x 8" photographic paper box. The box was wrapped in two layers of aluminum foil in order to keep out light, and placed in a freezer for 48 hours, after which the plate was developed. Since these X-ray films are sensitive to all wavelengths of white light, both loading and developing were done in darkness. Transferrin was observed on the X-ray film as dark zones against a grey background. Usually some Fe^{59} did not migrate, so that the origin could be readily recognized.

Adenosine deaminase

Adenosine deaminase was examined in harbor seals and humans by both starch gel and acrylamide gel electrophoresis using the continuous phosphate buffer system (pH 6.5) of Spencer, Hopkinson and Harris (1968). Undiluted red cell haemolysate samples of from five to twenty µl were electrophoresed for three hours at 300 volts. Adenosine deaminase zones were visualized in human red cell haemolysates using a stain containing adenosine, nucleoside phosphorylase (Boehringer), xanthine oxidase (Boehringer), tetrazolium salt MTT and phenazine methosulphate buffered at pH 7.5 and applied in an agar overlay. The composition of the stain mixture, and the reaction sequence are described by Spencer et al. (1968). No activity was detected in samples from 13 P. v. largha and nine P. v. richardsi that included eight adults, four subadults and ten pups.

Amylase

Amylase was examined in harbor seal serum and in both serum and saliva of humans by acrylamide gel electrophoresis using the Tris-citrate buffer system (pH 7.4) used for the examination of haem-binding proteins. Five and ten µl serum samples diluted with an equal volume of sucrose solution, and five µl samples of saliva that had been centrifuged and diluted with an equal volume of sucrose solution, were electrophoresed for two hours at 300 volts. Gels were stained following the technique of Boettcher and de la Lande (1969), which utilizes boiled potato starch and an iodine-potassium iodide solution. Starch concentrations of 0.2, 0.5 and 1.0% were used.

Amylase activity was detected in human serum and saliva samples but not in six P. v. richardsi and two P. v. largha serum samples. McDermid et al. (1972) likewise were unable to detect serum amylase activity in 42 Mirounga leonina. They attributed this inactivity to the age of their serum samples (more than one year). In this study, the P. v. richardsi samples were more than a year old and those from P. v. largha were only two months old, indicating that to examine serum amylase activity in pinnipeds, fresh serum samples should be used.

Postalbumin

A series of postalbumin zones was frequently visible on gels electrophoresed to display transferrins, but they were too faint to be typed routinely. Resolution of these zones was improved by applying 3 µl of serum diluted 1:1 with sucrose solution to the gels, and changing the gel buffer to Tris-citric acid at pH 6.6 (.01 M Tris, .006 M citric

acid). Then other faint postalbumin zones appeared, that were also difficult to type. Because of this, and because several different proteins may be incorporated within the ill-defined term "postalbumin", these zones were not considered satisfactory for routine examination.

Storage and photography of gels

After staining and inspection, acrylamide gels were placed in "Whirl-Paks", and starch gels were placed in "Saran Wrap" for storage at 4°C. Acrylamide gels shrank to about half their original size after a year of such storage, but could be readily enlarged by soaking in water for several hours. Gels were photographed using transmitted light (acrylamide gels) or reflected light (some starch gels). Starch gels which were to be stained for peptidase activity were sliced, laid on a 23 x 14 cm sheet of clear plastic with the cut surface uppermost, and the agar overlay containing the stain mixture poured on top. These gels could then be photographed by transmitted light.

V. NORTH PACIFIC HARBOR SEALS

V. I. Results

Esterase

Three serum esterase zones were observed migrating towards the anode (Fig. 6). The fastest had the same mobility as serum albumin, but was too faint for routine typing. The two slower zones had migration rates of 0.13 and 0.18 relative to albumin. All animals possessed the 0.18 esterase zone; the presence or absence of the 0.13 esterase zone appeared to be related to age. The age distribution of these zones among the 220 animals examined is shown in Figure 7. The 0.13 esterase zone was present in all animals older than 2-1/2 months. Its presence in some Port Heiden pups and its absence from the Tugidak series correlate with the greater average age of the former series. There was no variation in the mobility of the 0.18 esterase zone in 220 animals (Table 5).

Four esterase zones were observed when red cell haemolysates were examined by the same technique. The two slower zones corresponded in mobility to the 0.13 and 0.18 serum esterase zones. The other two were not sufficiently well resolved for routine typing.

Haemoglobin

Two anodal haemoglobin zones were observed at pH 8.5, the faster of which stained more intensely (Fig. 8). No variation was observed in the mobility of these zones in 194 animals (Table 5). Nor was any ontogenetic variation noted: haemoglobin of a full-term foetus and

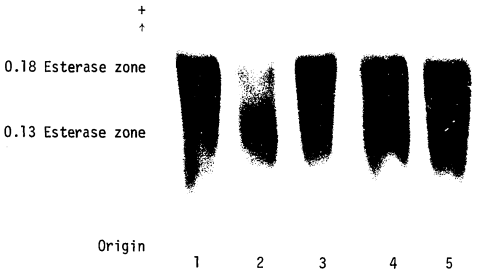

Figure 6. Starch gel electrophoretogram of Phoca vitulina richardsi sera, showing esterase zones. The 0.18 zone is present in all animals; the 0.13 zone is only present in samples 2 and 4. The samples are:

1. Izembek Lagoon 6; full-term foetus.
2. Izembek Lagoon 5; adult.
3. Izembek Lagoon 7; newborn.
4. Izembek Lagoon 4; subadult.
5. Izembek Lagoon 8; newborn.

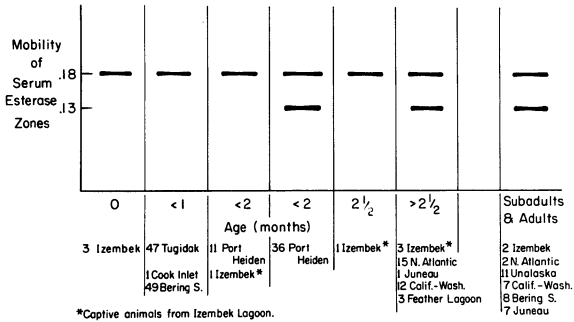

Figure 7. Age distribution of esterase zones in serum samples from Phoca vitulina. The number of animals examined is indicated.

TABLE 5. Numbers of Phoca vitulina examined electrophoretically.

Locality	Ester-ase++	Haemo-globin	Hapto-globin	Haemo-pexin	LDH	Pepti-dase	Trans-ferrin
P. v. richardsi							
Tugidak Island	47	43	38	47	47	41	47
Port Heiden	47	45	45	48	48	45	48
Izembek Lagoon	10+	11+	8	11+	11+	5	11+
Cook Inlet	1	0	0	0	1	0	1
Unalaska Island	11	0	12	12	12	0	13
Juneau	8	0	8	8	8	0	8
California-Washington	19+	21+	24+	24+	24+	18+	24+
P. v. concolor							
North Atlantic	17	16	17	17	17	16	17
P. v. largha							
Feather Lagoon	3	3	3	3	3	0	3
Bering Sea ice pack	57*	55*	23+	65*	66*	55*	62**
	220	194	178	235	237	180	234

+Includes 1 mother-pup pair.
*Includes 6 mother-pup pairs.
**Includes 4 mother-pup pairs.
++Zone with mobility 0.18 relative to albumin.

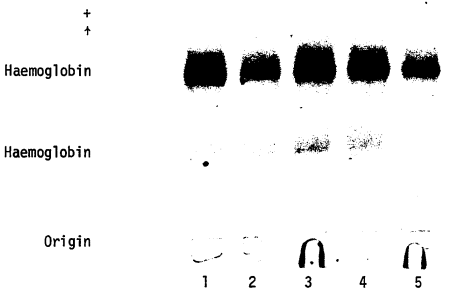

Figure 8. Acrylamide gel electrophoretogram of Phoca vitulina red cell haemolysates, showing haemoglobin zones. The samples are:

1. Bering Sea 4607.
2. Port Heiden 17.
3. Bering Sea 4627.
4. Tugidak Island 47.
5. Bering Sea 4639.

fourteen newborn pups was identical with that of other harbor seals.

Haptoglobin

Five zones showing peroxidase activity were observed (Fig. 9). Four migrated to the anode, and have been designated H1 to H4 in order of decreasing mobility. The cathodal zone, H5, was frequently diffuse in appearance. Both it, and a diffuse blur which formed the background to H4, had the same mobility as free haemoglobin.

Zones H3 and H4 are considered to be complexes of the serum protein haptoglobin with haemoglobin. Their identification was aided by the addition of increasing amounts of haemolysate to serum samples. This is illustrated for three subspecies of harbor seal in Figure 9. In a serum sample that was not visibly haemolysed, both zones were usually faint. As haemolysate was added to the serum, H3 disappeared, while H4 became more intense, until finally unbound haemoglobin appeared. Zone H3 and free haemoglobin were not observed together in the same sample. These observations are consistent with the model that the haptoglobin molecule of harbor seals can combine with either one or two units of haemoglobin, as originally proposed for human haptoglobin by Laurell and Nyman (1957), and Allison and ap Rees (1957), on the basis of similar experiments. It is therefore concluded that H3 is unsaturated haptoglobin, and H4 is haptoglobin saturated with haemoglobin. Recently, Nagel and Gibson (1971) have deduced from kinetic studies that human haptoglobin (type 1-1) contains two independent pairs of binding sites, one pair for each haemoglobin dimer ($\alpha\beta$), and that haptoglobin will combine only with haemoglobin dimers.

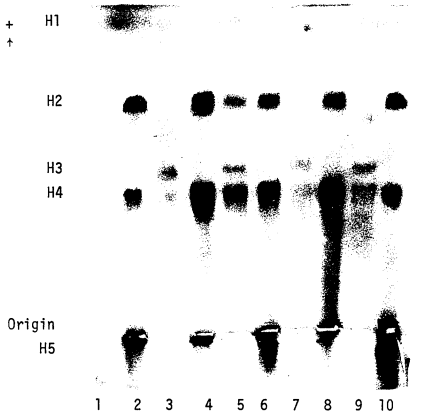

Figure 9. Starch gel electrophoretogram of Phoca vitulina sera, showing peroxidase-active zones. The samples are:

1. North Atlantic 6; serum.
2. North Atlantic 6; serum with added haemolysate.
3. California-Washington 1; serum.
4. California-Washington 1; serum with added haemolysate.
5. Bering Sea 4624; serum.
6. Bering Sea 4624; serum with added haemolysate.
7. Unalaska Island 12; serum.
8. Unalaska Island 12; serum with added haemolysate.
9. Tugidak Island 1; serum.
10. Tugidak Island 1; serum with added haemolysate.

Haptoglobin was observed in all adults, subadults and pups older than two months, but could not be detected in 55 pups younger than one month (Table 6), including the full-term foetus and two newborn pups from Izembek Lagoon and 11 newborn largha. Nor could haptoglobin be detected in another three pups from Port Heiden that were younger than two months, and may have been younger than one month. In many pups haptoglobin stained less intensely than in adults; these are referred to as "faint" in Table 6. Haptoglobin was present in "normal" concentration in a smaller proportion of the Tugidak series than in the older Port Heiden series. Thus the presence or absence of haptoglobin in an animal is related to its age. The youngest known-age harbor seal in which haptoglobin was observed was a four-week-old pup from Izembek Lagoon which was kept in captivity at the Institute of Arctic Biology. However, several of the unweaned Tugidak and Bering Sea pups which displayed haptoglobin could have been less than four weeks old.

No variation was observed in the mobility of the haptoglobin complex of 178 harbor seals (Table 5).

Haemopexin

Zone H1 had the same mobility as albumin, and so was considered to be methaemalbumin, the complex of haematin with albumin. It stained weakly and could not be typed routinely. The serum protein haemopexin binds haematin more strongly than does albumin (Manwell and Baker, 1970). Thus H2, which was more intense than H1, was considered to be the haemopexin-haematin complex.

Haemopexin was observed in all animals except the starvling pup

TABLE 6. Haptoglobin staining intensity among *Phoca vitulina* pups.

Locality	Age	Haptoglobin staining status: normal*	faint**	not detected	Number of pups examined
P. v. richardsi					
Tugidak Island	<1 month	16	22	9	47
Port Heiden	<2 months	39	6	3	48
Izembek Lagoon	<1 week	0	0	3	
	>1 month	6	0	0	9
Cook Inlet	<1 month	0	0	1+	1
Juneau	5 months	1	0	0	1
California-Washington	5 months	16	0	0	16
P. v. concolor					
North Atlantic	3 months	15	0	0	15
P. v. largha					
Feather Lagoon	5 months	3	0	0	3
Bering Sea ice pack	<1 month	7	8	42	57

*The staining intensity observed in adults.
**Staining intensity obviously less than that in adults.
+Nor was haemopexin observed in this animal.

from Cook Inlet (Table 5). In a small number of pups it was present in much lower concentrations than in adults: four from Bering Sea, two of which were newborn; six from Tugidak Island; and three from Izembek Lagoon, including the full-term foetus and a newborn pup. Thus haemopexin must appear in serum before haptoglobin.

No variation was observed in the mobility of the haemopexin complex of 235 harbor seals (Table 5).

Lactate dehydrogenase

Five anodal LDH zones were observed in sera of all animals (Fig. 10). Zones 1 and 2 stained less intensely than the others, indicating a preponderance of M units in serum. No variation was observed in the mobility of these zones in a total of 237 animals (Table 5). Neither was any ontogenetic variation noted: the LDH staining pattern of the full-term foetus and 14 newborn pups appeared identical with that of other harbor seals.

Peptidase

A single peptidase zone was observed migrating to the anode (Fig. 11). It will be referred to as peptidase B as it corresponds to the human peptidase in both substrate specificity and relative electrophoretic mobility (Lewis and Harris, 1967). No variation was observed in the mobility of this zone in 180 animals (Table 5). Likewise, no ontogenetic variation was observed.

Other peptidase zones were observed when the dipeptides glycyl-leucine, leucyl-alanine and phenylalanyl-proline were used as substrate. They corresponded, respectively, to human peptidases A, C and D of Lewis

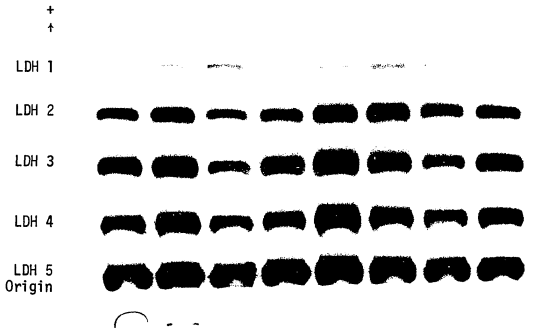

Figure 10. Acrylamide gel electrophoretogram of Phoca vitulina sera, showing LDH zones. The samples are:

1. P. v. richardsi; Unalaska Island 17.
2. P. v. largha; Bering Sea 4612.
3. P. v. richardsi; Izembek Lagoon 107.
4. P. v. richardsi; Cook Inlet 57.
5. P. v. concolor; North Atlantic 6.
6. P. v. richardsi; California-Washington 25.
7. P. v. richardsi; Port Heiden 47.
8. P. v. richardsi; Tugidak Island 10.

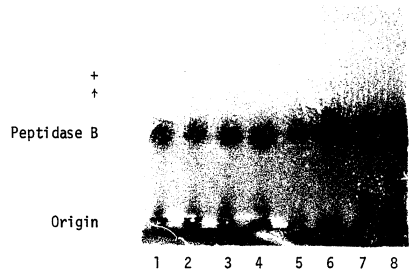

Figure 11. Starch gel electrophoretogram of Phoca vitulina red cell haemolysates, showing peptidase B zones. The samples are:

1. P. v. richardsi; California-Washington 5.
2. P. v. richardsi; Port Heiden 46.
3. P. v. richardsi; California-Washington 6.
4. P. v. richardsi; Port Heiden 23.
5. P. v. concolor; North Atlantic 3.
6. P. v. richardsi; California-Washington 3.
7. P. v. richardsi; Tugidak Island 47.
8. P. v. richardsi; California-Washington 12.

and Harris (1967), but stained too faintly for routine typing.

Transferrin

Two strongly staining transferrin zones were observed on acrylamide gels and on autoradiographs (Figs. 12 and 13). They have been designated TfA and TfB in order of decreasing electrophoretic mobility. Associated with these zones were up to four weakly-staining (minor) zones, two migrating more slowly than TfB, one faster than TfB and one faster than TfA. Although they were not observed on autoradiographs, such minor zones are common in many species (Manwell and Baker, 1970) and so it was assumed that they were transferrin. The complexity of a particular transferrin phenotype is due to the various number of sialic acid residues attached to the transferrin molecule (Chen and Sutton, 1967). Of the 234 animals examined for transferrin (Table 5), two were TfAB and the others were TfB.

In every species for which data are available, transferrin variation is determined in a simple Mendelian manner (Lush, 1966). Hence TfB harbor seals were assumed to be homozygotes ($\underline{Tf}^B/\underline{Tf}^B$), and TfAB seals were assumed to be heterozygotes ($\underline{Tf}^A/\underline{Tf}^B$). The family data typed for transferrin (six mother-pup pairs) did not contradict this assumption, as they were all TfB.

The TfAB animals were from Tugidak Island (one of 47 animals) and Port Heiden (one of 48 animals). The estimate of the frequency of the $\underline{Tf}^A$ allele at these locations is thus 1/94 and 1/96, both slightly greater than 0.01. Consequently, these populations can be considered polymorphic by the 0.99 criterion, but not by the 0.95 criterion.

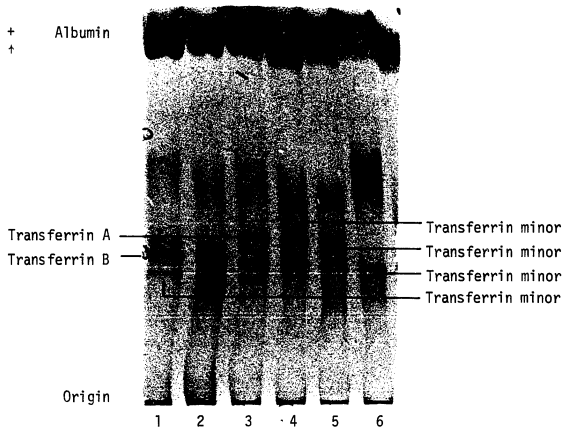

Figure 12. Acrylamide gel electrophoretogram of Phoca vitulina richardsi sera, showing transferrin zones. Beside the strongly staining (major) zones (TfA and TfB) that determine transferrin phenotype, there are four weakly-staining (minor) zones: one faster than TfA; one faster than TfB; and two slower than TfB. The samples, with their transferrin phenotypes, are:

1.	Tugidak Island 51	TfB
2.	Unalaska Island 18	TfB
3.	Tugidak Island 44	TfAB
4.	Port Heiden 2	TfAB
5.	Port Heiden 19	TfB
6.	Cook Inlet 57	TfB

Figure 13. Autoradiograph of Phoca vitulina sera with added Fe^{59}, showing transferrin zones. The samples, with their transferrin phenotypes, are:

1.	Port Heiden 2	TfAB
2.	North Atlantic 15	TfB
3.	Tugidak Island 44	TfAB

Caution was required when typing transferrins, for after TfB serum samples had been thawed and refrozen several times, the intensity of the fast minor zone (with the same mobility as the TfA major zone) increased, and such samples could be mistaken for TfAB. The two presumed heterozygotes consistently displayed the same pattern, so that there was no reason to suspect that their TfA zone was an artifact. No ontogenetic variation was observed.

Genetic variability of harbor seal populations

Estimates of genetic variability in eight harbor seal populations are given in Table 7. In estimating the proportion of polymorphic loci, the 0.99 criterion for polymorphism was used. The number of loci examined was estimated as follows: two each for haptoglobin and LDH, three for haemoglobin (two loci for each haemoglobin zone, one of which is common to both zones, as both Seal (1969) and Suderman, et al. (1973) have speculated that α chains of the two pinniped haemoglobins are identical, and that the non-α chains differ), and one each for the other proteins.

For the six populations of P. v. richardsi the mean values of P and H are 0.030 and 0.001, respectively. For all eight populations of P. vitulina they are 0.023 and 0.001.

Genetic comparison of harbor seal populations

To measure degrees of genetic similarity among harbor seal populations, Rogers' coefficient, S (Rogers, 1972), has been used in order to permit comparisons with studies of other vertebrates. Paired combinations of populations involving either the Tugidak or the Port Heiden series, and another series, yielded S values of 0.999. All other combinations (including

TABLE 7. Estimates of genetic variability in populations of *Phoca vitulina*.

Population	Number of loci examined	Mean proportion of polymorphic loci per population (P)	Mean proportion of heterozygous loci per individual (H)	Number of alleles per locus per population
P. v. richardsi				
Tugidak Island	11	.091	.002	1.09
Port Heiden	11	.091	.002	1.09
Izembek Lagoon	11	0	0	1.0
Unalaska Island	7	0	0	1.0
Juneau	7	0	0	1.0
California-Washington	11	0	0	1.0
P. v. concolor				
North Atlantic	11	0	0	1.0
P. v. largha				
Bering Sea ice pack	11	0	0	1.0

that of Tugidak and Port Heiden) yielded values of 1.000.

V. 2. Discussion

Haptoglobin variation in harbor seals and other species

The fact that some animals possessed an unsaturated haptoglobin zone in addition to the saturated haptoglobin zone found in all animals might be mistaken for a polymorphism with two phenotypes. Haptoglobin polymorphism has been found in only a few species apart from humans (e.g., ring-necked pheasants, Phasianus colchicus, Baker et al., 1966; hooded seals, C. cristata, Naevdal, 1966b; and rabbits, Chiao and Dray, 1969).

Two haptoglobin phenotypes have been observed in several species other than harbor seals: northern fur seals, C. ursinus (Blumberg, Allison and Garry, 1960), Arctocephalus sp. (Shaughnessy, 1970), southern elephant seals, M. leonina (Shaughnessy, in prep.), domestic dogs (Naik et al., 1971; Shaughnessy, unpub.), dingos, Canis familiaris (Shaughnessy, unpub.), several non-human primates (Blumberg, 1960) and the marsupial Marmosa mitis (Shifrine and Stormont, 1970). However, it was found that the faster haptoglobin zone could be made to disappear in the presence of excess haemoglobin in each case, except the last, where this was not attempted. Also, a third phenotype which would be expected if this was a genetical polymorphism was notably absent. Blumberg et al. (1960) suggested that the two phenotypes in northern fur seals were under genetical control, and since then several authors have referred to this as a haptoglobin polymorphism (e.g., Seal, Erickson, Siniff and Cline,

1971). Likewise, Shifrine and Stormont (1970) suggested that haptoglobin types in M. mitis were under genetical control. However, it seems more likely that, in each species (including harbor seals), the faster zone is merely haptoglobin that is not saturated with haemoglobin, and thus, that the haptoglobin molecules of each zone are in fact genetically identical. Koehn (1966) provided the latter explanation for the electrophoretic variation he observed in haptoglobin of some catostomid fish.

Paucity of genetic variation in harbor seal populations

When compared with estimates of the amount of genetic variation in other animals (Table 3 and page 60), estimates for the harbor seal populations are seen to be low, even allowing that seven of the 11 loci examined controlled non-enzymatic proteins. This paucity of variation also contrasts with the situation in other pinnipeds, for electrophoretic variation has been observed in all species that have been examined for at least four proteins, with the exception of M. angustirostris.

Situations which lead to a paucity of genetic variation in natural populations will be considered with respect to harbor seals. One possible cause is random fluctuations of gene frequency leading to fixation and extinction of alleles (genetic drift) when a population passes through a genetic "bottleneck," i.e., when its effective population size is reduced to about 100, or in populations occupying small islands or at extremities of the species range. The uniformity of blood proteins in another pinniped, M. angustirostris has been attributed to a genetic "bottleneck" (Bonnell, in litt., 1972), as the species was almost

exterminated during the nineteenth century (Bartholomew and Hubbs, 1960). Several investigations have demonstrated significantly reduced genetic variability in island and isolated populations that has been attributed to genetic drift: e.g., the population of Drosophila pseudoobscura at Bagotá, Colombia was much less variable than those from the main part of the species range in southwestern U.S.A. (Prakash, Lewontin and Hubby, 1969); in Peromyscus polionotus populations on Santa Rosa Island, H was about one third of that in populations on the Florida Panhandle (Selander et al., 1971); and in isolated cave populations of the fish, Astyanax mexicanus, H ranged from 0.00 to 0.08, while in surface-dwelling populations, it ranged from 0.08 to 0.14 (Avise and Selander, 1972). However, there is no evidence for past decimation of any harbor seal populations, and only one of the sampled populations of P. v. richardsi (that from Port Heiden) could be considered to be peripheral.

Another possible cause of the homogeneity of harbor seal populations is that normalizing or stabilizing selection has been acting to maintain genetic homogeneity, and the genome has become fixed for an optimal genotype from which only small changes are tolerated. A similar explanation was provided by Johnson et al. (1973) for the low variability in populations of rockfish (Sebastes sp.), that are among the least variable of populations, and like harbor seals, occupy an aquatic environment. However, it seems unlikely that homeostasis would act in harbor seals, but not in other pinnipeds.

A third possibility is that the harbor seal is a relatively recent

invader in the North Pacific, and that colonizers from the North Atlantic were few and genetically depauperate. Two hypotheses [discussed by Fay (1973)] have been proposed for the evolution of these seals. The one invoked above, originally due to Davies (1958), proposes that North Atlantic harbor seals migrated westward across northern Canada and into the Bering Sea during an interglacial period of the Pleistocene. During a later glacial period, those harbor seals in a sea ice habitat gave rise to P. v. largha.

The other, almost antithetical, hypothesis for the evolution of harbor seals has been proposed by McLaren (1966; 1973) and Chapskii (1969). In this scheme, an ancestor close to modern ringed seals gave rise to P. v. largha in the sea ice areas of the western North Pacific Ocean. Land-breeding P. vitulina later arose in ice-free areas of the North Pacific, and invaded the North Atlantic via the Canadian Arctic.

As populations of harbor seals from both oceans were found to be electrophoretically homogeneous and identical in this study, it was not possible to decide between these two evolutionary models. If the population from one ocean were shown to be considerably less variable than the other, then the former could confidently be described as the more recent arrival. However, the occurrence of fossils resembling P. vitulina from late Pliocene or early Pleistocene time from Oregon and California (Barnes, 1973) suggests that the paucity of variation in blood proteins of North Pacific harbor seals cannot be attributed to their recent invasion.

Small amounts of electrophoretic variation among blood proteins of

some populations has been attributed to the relatively homogeneous nature of their environment; e.g., subterranean populations of the mole rat, Spalax, in which H ranges from .018 to .056 (Nevo and Shaw, 1972) and a population of the deep sea pogonophoran, Siboglinum atlanticum in which P was .056 (Manwell, Baker, Southward and Southward, unpub., in Manwell and Baker, 1968). Since the harbor seal populations sampled in this study were from widely spaced localities, homogeneity of their blood proteins cannot be attributed to environmental homogeneity.

The homogeneity observed in blood proteins of North Pacific harbor seals is in marked contrast to their variation in coat color and pattern (p. 16), behavior (Burns et al., 1972) and ecology (Burns and Fay, 1973; Naito and Nishiwaki, 1973). Variation within subarctic species has been considered in an ecological context by Dunbar (1968). He contrasted the situation in tropical regions, where there is a vast number of readily discernible species, with that in higher latitudes where the amount of intraspecific variation increases with latitude, at least to the subarctic. He argued that the profusion of variation in temperate and subarctic waters is providing the raw material for species diversification.

Harbor seals of the North Pacific and Bering Sea are located in these temperate and subarctic regions, and judging by their variations in external appearance and habitat requirements, it would seem that they too are diversifying, as Dunbar predicts. However, this overt diversification is not repeated as cryptic diversification in blood proteins, suggesting that not all of the genome is subject to change at the same rate. A similar lack of parallelism between morphological

(and also chromosomal) differentiation on the one hand, and electrophoretic differentiation on the other, has been observed in Thomomys by Patton, Selander and Smith (1972).

Similarity of harbor seal populations

Although two populations of P. v. richardsi were polymorphic for transferrin, while P. v. largha and P. v. concolor were homogeneous, the transferrin variant was so uncommon that this character could not satisfactorily be used to distinguish between subspecies. Nor could other protein markers be used for this purpose, as they were all homogeneous.

Estimates of S for harbor seal populations are higher than those for other vertebrate populations (reviewed by Avise and Selander, 1972), and in particular are much higher than those for interspecific comparisons. On the basis of these comparisons, the ice-breeding North Pacific harbor seal cannot be considered specifically distinct from P. v. richardsi. Nor can the population of harbor seals in the Aleutian Islands (referred to as P. insularis [=stejnegeri] by Belkin et al., 1969) be recognized as specifically distinct from P. v. richardsi. However, it must be stressed that only a portion of the genome has been sampled in this study, so that these results should not be considered alone, but with evidence based on other characters. Such studies are proceeding (Naito and Nishiwaki, 1972b; Burns and Fay, 1973).

Another study of similarity of eastern North Pacific harbor seal populations was made by Stutz (1967). He divided 247 skins from seven localities into three classes (black, common and muddy) and claimed that

significant differences in the distribution of these phenotypes indicated genetical differences between populations. Although his method of analysis (an analysis of variance) was inappropriate (S. J. Harbo, voc. comm.), a heterogeneity χ^2 test shows that the two classifications, pelage type and location, are strongly associated ($\chi^2_{10} = 33.9$, $P<0.001$; with the locations Skeena and Juneau grouped to prevent the inclusion of small numbers of observations). However, there is no indication of the extent to which pelage type is controlled by genetical and environmental factors. Further, harbor seal pelages are notoriously difficult to classify and Stutz did not indicate the reliability or repeatability of his classification. Thus, Stutz's conclusions of restricted gene flow between populations of the harbor seal along the Washington, British Columbia and southeastern Alaska coasts should be treated with caution until more is known of the inheritance of pelage types.

Populations of ice-breeding and land-breeding harbor seals of the North Pacific have generally been considered reproductively separated because of temporal isolation and, at least in eastern and central Bering Sea, spatial isolation. However, a close examination of the pertinent literature, and some recent extensions of known breeding range of P. v. richardsi suggest that introgression is possible. The ranges of richardsi and largha approach in eastern Bering Sea where largha breeds two to three months before richardsi (p. 17). Few data are available on the length of time that male harbor seals in this area are in breeding condition. In British Columbia, Bigg (1969b) found that epididymes of males contained sperm from March to November, even though he considered that most matings

occurred in September [although Figure 1 in Bigg (1969a) suggests most matings occur earlier]. If male richardsi in eastern Bering Sea were potent even three months before their breeding season commenced, they would be capable of fertilizing female largha. Similarly, if male largha were potent for two to three months after their breeding season (as are male richardsi in British Columbia), they would be capable of fertilizing female richardsi. The most likely areas in eastern Bering Sea for such inter-subspecific matings to occur are on the Pribilof Islands and in Bristol Bay where the known breeding ranges of richardsi and largha meet and may overlap. More knowledge of the life history of both forms of the harbor seal in this area is obviously necessary to determine if they are in close proximity during times when cross-fertilizations are possible. Conceivably, overlaps in the ranges of breeding animals occur often enough to permit introgression and maintenance of the genetic similarity of the two forms. In the southern Kuril Islands, Belkin et al. (1969) reported that largha and stejnegeri were sympatric, but believed that they were reproductively isolated, as their pupping seasons were two months apart. This difference is shorter than that in eastern Bering Sea, but as there is no information on the length of time that males of either of these forms are potent, it is difficult to speculate on the possibility of introgression between them.

Introgression between richardsi and stejnegeri is even more likely to occur, for as discussed earlier (p. 56), no isolating mechanisms have been suggested for these taxa, and even the western and eastern limits of their respective ranges are doubtful.

Evolution of North Pacific harbor seals

It is possible to obtain some information on the time since divergence of P. v. richardsi and P. v. largha based on the absence of protein variation between them, and on estimates of the rates of amino acid substitutions in proteins. The latter vary between proteins: rates for 15 proteins in the mammalian line of descent based on comparisons of amino acid sequences have been reported by McLaughlin and Dayhoff (1972). Their mean value (2.4×10^{-9} substitutions per amino acid site per year) will be used for the seven harbor seal proteins, as only one of them (haemoglobin) was included in this study. Since electrophoresis detects only substitutions that result in a change in net charge of a protein, which, as noted on page 62, represents approximately 29% of all substitutions, it can be predicted that an average of $0.29 \times 2.4 \times 10^{-9}$ amino acid substitutions occur per amino acid site per year that can be detected electrophoretically.

The number of amino acid sites in the harbor seal proteins examined in this study is not known; indeed, it is only known for one of these proteins in humans, viz. haemoglobin, which has 141, 146 and 146 sites in its α, β and γ chains, respectively (Dayhoff et al., 1972). The number of amino acid sites in the other proteins can be estimated from their molecular weights (Table 8), indicating that a total of 3,820 amino acid sites was examined.

Now, $0.29 \times 2.4 \times 10^{-9}$ amino acid substitutions causing a change in charge occur per amino acid site per year, so that one such change would

TABLE 8. Estimates of the number of amino acid sites in the proteins examined in Phoca vitulina.

Protein	Molecular weight	Reference	Estimated no. of amino acid sites*	Comments
Esterase	70,000	Tashian (1969)	640	Human. A minimum estimate.
Haemoglobin	--	Dayhoff et al. (1972)	430	Human. Based on amino acid sequences.
Haptoglobin	84,000	Wakes, Alfsen and Cittanova (1967)	380	Human. A dimer, M. Wt. of the two polypeptide chains used.
Haemopexin	65,000	Witz and Gross (1965)	590	Mouse.
LDH	136,000	Giblett (1969, 521)	620	Human. A tetramer, M. Wt. of the two polypeptide chains used.
Peptidase B	54,000	Lewis and Harris (1969)	490	Human.
Transferrin	78,000	Greene and Feeney (1968)	670	Human, rabbit and chicken. The carbohydrate portion (5.5% according to Giblett, 1969, 127) deducted.

*Obtained from the molecular weight using 110 as the average molecular weight of an amino acid (Smith, 1966).

be expected in 3,820 sites in $\frac{1}{0.29 \times 2.4 \times 10^{-9} \times 3{,}820}$ years

$= 376{,}000$ years

$\doteq 400{,}000$ years.

That no such substitutions were detected in proteins of P. v. richardsi and P. v. largha indicates that they diverged more recently.

An attempt will now be made to amalgamate evidence from electrophoretic data and fossils with current theories on the paleogeography and climatic history of Beringia (Hopkins, 1972) in order to elucidate the evolutionary history of North Pacific harbor seals.

Ancestors of modern Phoca were present in the eastern North Pacific by late Pliocene or early Pleistocene (Barnes, 1973), or even earlier, according to McLaren (1973), who proposed that Phoca evolved there from Pusa which is represented by a fossil radius from mid-Pliocene Alaska [Yakataga Formation, Gulf of Alaska (Repenning, in litt., 1972)]. If McLaren's theory that the land-breeding form arose from a largha-like derivative of Pusa is correct, then the divergence of the two forms of harbor seal may have taken place during a warm, interglaciai period when the sea ice habitat was restricted, e.g., during the last (Sangamon) interglaciation which occurred from 100,000 to 70,000 years ago. The Bering Seaway was open during that period, providing the opportunity for the new form to invade the Arctic Ocean and spread to the North Atlantic. Another, briefer, opportunity occurred from 30,000 to 26,000 years ago during a warm period of the Wisconsin glaciation (Hopkins, 1967, 464).

On the other hand, the late Pliocene -- early Pleistocene Phoca

fossils from California (Barnes, 1973) are of the heavy-jawed richardsi-stejnegeri type, suggesting that the North Pacific harbor seal population was founded by land-breeding types, long before the 100,000 year divergence. Conceivably, these came from the North Atlantic Ocean, as proposed by Davies (1958), and the ice-breeding population arose from them much later, probably during a cold period when ice-cover (both sea ice and glacial ice shelves) was extensive. This might have occurred during the last (Wisconsin) glaciation which began about 70,000 years ago, or during the penultimate (Illinoian) glaciation, about 100,000 years ago and earlier. Since the Illinoian is believed to have been the most extensive of the middle and late Pleistocene glaciations, it is more likely to have provided the conditions to produce divergence of an ice-breeding form.

The two land-breeding taxa, P. v. richardsi and P. v. stejnegeri, are more similar to each other than either of them is to P. v. largha (p. 56); this suggests that they are either segments of an imperfectly known polytypic species, or two slightly divergent forms that were separated from each other for a relatively short time, perhaps during a mid- to late-Pleistocene glacial period, after which their ranges reconverged. Such a separation might have occurred during the peak of the Wisconsin glaciation (20,000 to 15,000 years ago) when sea level was at a minimum, the abyssal basin of the Bering Sea was covered with sea ice at least during winter, and there was extensive glacial ice shelving along the Aleutian Islands, Alaska Peninsula and eastern Kamchatka. At that time, the land-breeding harbor seals might have

shifted southward along the eastern and western North Pacific seaboards, becoming isolated from each other.

Other approaches to the biochemical identification of populations

Electrophoresis of milk proteins may provide a more useful method for the differentiation of harbor seal populations. The main role of the non-enzymatic milk proteins is thought to be as a source of amino acids for the nursing young (Jenness, 1970) with the exception of α-lactalbumin which is involved in lactose synthesis. Thus, since the amino acid sequence of milk proteins should be subject to fewer restrictions than are blood proteins which perform specific functions, there should be more opportunity for variability in milk proteins. This is supported by observations of milk and blood proteins in bears (Jenness, Erickson and Craighead, 1972) and in M. leonina (Shaughnessy, in prep.).

The most direct means of comparing populations is with amino acid sequences of individual proteins. This is a much more time-consuming, and therefore more expensive, approach than the electrophoretic scanning used in this study. Consequently, usually only one, or at most a few, individuals of each group are compared, and so the possibility that polymorphism exists in the protein being examined is ignored.

The amino acid sequence of myoglobin of Bering Sea harbor seals was compared by Nauman (1970) with that found for Maine coast animals (P. v. concolor) by Bradshaw and Gurd (1969). No differences could be found between the amino acid sequence of myoglobins of these two forms of the harbor seal. However, since Nauman also found that the amino

acid sequence of myoglobin of three other phocids (P. hispida, E. barbatus and L. weddelli) appeared to be identical with that of P. vitulina, the uniformity of myoglobin of the two harbor seal subspecies is not surprising.

Myoglobin appears to be one of the more conservative of the proteins whose amino acid sequences have been determined: amino acid substitutions have been observed in myoglobin at a rate of 1.3×10^{-9} substitutions per amino acid site per year, which is considerably less than the average rate for 15 proteins of 2.4×10^{-9} (McLaughlin and Dayhoff, 1972). Since the most variable proteins are the fibrinopeptides, with an amino acid substitution rate of 9.0×10^{-9}, their amino acid sequences are most likely to illustrate differences and affinities between taxa.

VI. ELECTROPHORETIC COMPARISONS OF BERING SEA PINNIPEDS

The proteins haemoglobin, haptoglobin, haemopexin and transferrin were also electrophoresed in other Bering Sea pinnipeds: ribbon seal, Histriophoca fasciata; bearded seal, Erignathus barbatus; walrus, Odobenus rosmarus; and Steller sea lion, Eumetopias jubata.

VI. 1. Results

Haemoglobin

Two anodal haemoglobin zones were observed in each species (Fig. 14). The faster zone had the same mobility as that of harbor seals. The slower zone of ribbon seals, bearded seals and the Steller sea lion also had the same mobility as that of harbor seals (Table 9), and was fainter than the fast zone. In the walrus, the two zones were much closer together, and of similar staining intensity.

Haptoglobin and haemopexin

These proteins were examined only in bearded seals and ribbon seals. The former possessed two intensely-staining haem-binding zones. They were not positively identified, but by analogy with the haem-binding zones of harbor seals, were judged to be haemopexin and haptoglobin (Fig. 15). Each ribbon seal posssessed a zone with mobility similar to harbor seal haemopexin, but a zone in the haptoglobin position was obvious only in the subadult; it could not be seen in the newborn pup, and was barely visible in another pup (Fig. 16). Mobilities of these zones in the three species are set out in Table 10. Distances were

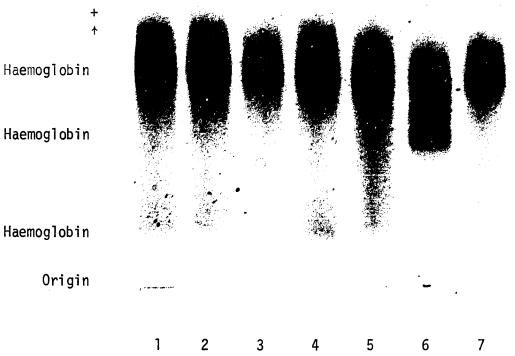

Figure 14. Acrylamide gel electrophoretogram of pinniped red cell haemolysates, showing haemoglobin zones. The samples are:

1. Phoca vitulina concolor; North Atlantic 3.
2. P. v. richardsi; California-Washington 7.
3. P. v. largha; Bering Sea 4624.
4. Histriophoca fasciata; Hist-1.
5. Erignathus barbatus; Erig-2.
6. Odobenus rosmarus; Odob-1.
7. Eumetopias jubata; Eum-1.

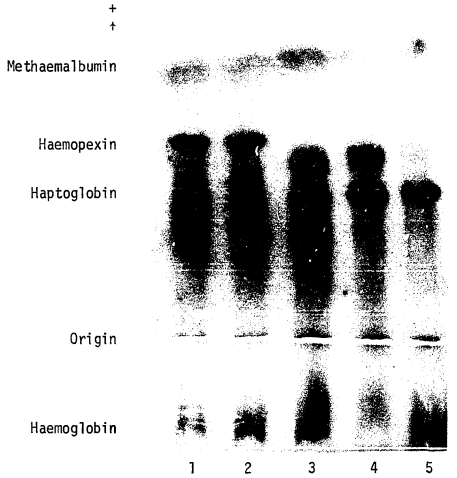

Figure 15. Starch gel electrophoretogram of Phoca vitulina and Erignathus barbatus sera, with added red cell haemolysate, showing peroxidase-active zones. The samples are:

1. P. v. largha; Bering Sea 4603.
2. P. v. largha; Bering Sea 4604.
3. E. barbatus; Erig-1.
4. E. barbatus; Erig-2.
5. E. barbatus; Erig-3.

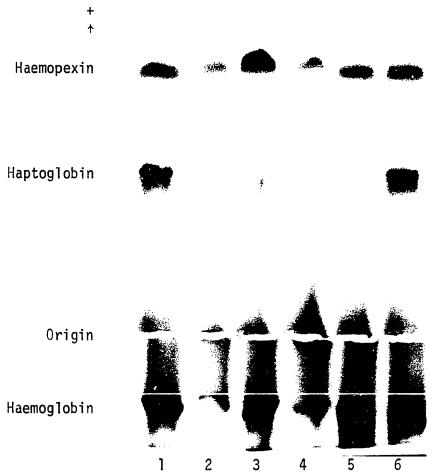

Figure 16. Starch gel electrophoretogram of Phoca vitulina and Histriophoca fasciata sera, with added red cell haemolysate, showing peroxidase-active zones. The samples are:

1. P. v. richardsi; Izembek Lagoon 107.
2. H. fasciata; Hist-1.
3. H. fasciata; Hist-2.
4. H. fasciata; 4605.
5. P. v. largha; Bering Sea 4636.
6. P. v. richardsi; Port Heiden 24.

TABLE 9. Migration of haemoglobin zones in several Bering Sea pinnipeds.*

Genus	Sample no. (in Fig. 14)	Fast zone	Slow zone	Relative mobility of slow zone
Phoca	3	2.98	0.86	0.29
Histriophoca	7	2.85	0.77	0.27
Erignathus	8	2.75	0.71	0.26
Odobenus	9	2.76	2.02	0.73
Eumetopias	10	2.74	0.78	0.28

*Mean of three measurements, in cm.

TABLE 10a. Migration of haem-binding proteins in Phoca vitulina and Erignathus barbatus.*

	Phoca	Erignathus
Haptoglobin	3.02	3.20
Haemopexin	4.41	3.98

*Mean of three measurements, in cm.

TABLE 10b. Migration of haem-binding proteins in Phoca vitulina and Histriophoca fasciata.*

	Phoca	Histriophoca
Haptoglobin	2.89	2.77
Haemopexin	4.76	4.92

*Mean of three measurements, in cm.

measured from the origin with dial-reading calipers. Only samples in adjacent or nearby slots on the same gel were used for these measurements.

Transferrin

Transferrins of ribbon seal and a bearded seal are compared with that of a harbor seal in Fig. 17.

Two transferrin phenotypes were observed in ribbon seals: one with a single zone (TfF, in animal 4605); the other with this zone and a slow migrating zone (TfSF, in animal Hist-1). Transferrin could not be typed in two other ribbon seals. After repeated thawing and freezing of the serum sample from animal Hist-1, a third slower zone appeared, but the single zone of 4605 remained unaltered (Fig. 18). Each of the three zones of Hist-1 bound radioactive iron (Fig. 19). Such deterioration of transferrin zones has been attributed to the cleavage of sialic acid residues by neuraminidase (Blumberg and Warren, 1961). It has also been reported for sheep transferrins (Ashton and Ferguson, 1963).

Transferrin of bearded seals was not identified by autoradiography. An intensely-staining zone with the same mobility as TfB of harbor seals was observed, and this, together with a faster-migrating minor zone, was assumed to be transferrin.

The observed variation in transferrin of ribbon seals may represent genetic polymorphism. However, the appearance of a third transferrin zone in the TfSF phenotype suggests, conversely, that some of the variation was an artifact due to deterioration in storage.

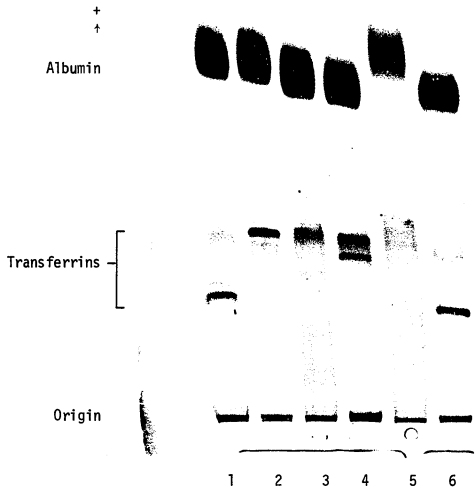

Figure 17. Acrylamide gel electrophoretogram of pinniped sera, showing transferrin zones. The samples are:

1. Erignathus barbatus; Erig-1.
2. Histriophoca fasciata; 4605.
3. H. fasciata; Hist-2.
4. H. fasciata; Hist-1.
5. H. fasciata; 4655.
6. Phoca vitulina largha; Bering Sea 4656.

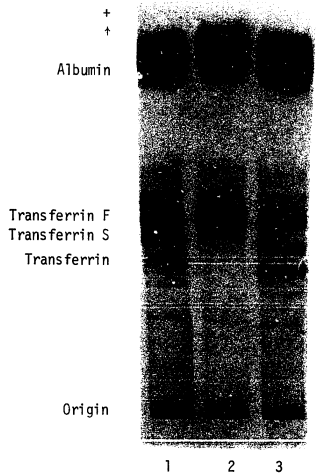

Figure 18. Acrylamide gel electrophoretogram of Histriophoca fasciata sera, showing transferrin zones S, F and a slower one caused by deterioration. Samples 1 and 3 are also shown in Fig. 19. The samples, with transferrin phenotypes, are:

1. Hist-1 TfSF
2. 4605 TfF
3. Hist-1 TfSF

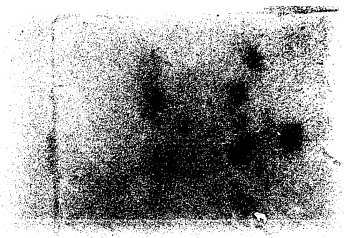

Figure 19. Autoradiograph of Histriophoca fasciata serum with added Fe^{59}, showing transferrin zones. The other slice of this gel, stained with amido black, is shown in Fig. 18. The samples are both Hist-1, with transferrin phenotype SF, and correspond to samples 1 and 3 in Fig. 18.

VI. 2. Discussion

Haemoglobin ontogeny in phocids

Ontogenetic sequences of haemoglobin in vertebrates have been reviewed by Manwell and Baker (1970). The best known occurs in man and few are known among other mammals. With the exception of newborn Pagophilus groenlandicus, in which the haemoglobin pattern was identical with that of adults (Naevdal, 1966a), haemoglobin of foetal and newborn pinnipeds does not appear to have been examined electrophoretically previous to this study. The haemoglobin pattern of 14 newborn harbor seals and a full-term foetus was identical with that of adults. Likewise no differences were observed between haemoglobin of newborn and subadult ribbon seals, nor between newborn and adult bearded seals.

Time of appearance of haptoglobin in young pinnipeds

Haptoglobin has not been detected in newborn young of some mammalian species, e.g., humans (Galatius-Jensen, 1957) and chimpanzees Pan troglodytes (Planas, 1970). Among the North Pacific harbor seals examined in this study, haptoglobin could not be detected in 58 pups less than a month old, and was noticeably weaker in another 36 young pups (Table 6). Nor could haptoglobin be detected in a newborn ribbon seal, while it was only weakly expressed in an older pup. On the other hand, haptoglobin was present, and stained intensely, in a bearded seal pup one to three days old. In pups of another phocid, C. cristata, Naevdal (1966b) found that haptoglobin zones were weaker than in adults.

Pinnipeds are more advanced in development at birth than most mammals, the phocids more so than the otariids (Laws, 1959). Two of

the largest at birth are the southern elephant seal, M. leonina (Bryden, 1972), and the bearded seal (Burns, 1967).

In addition to the finding of haptoglobin in a young bearded seal, strong haptoglobin zones were also found in four southern elephant seal pups sampled on the day of their birth (Shaughnessy, 1968). In a study of the otariids, A. pusillus and A. forsteri, some pups as old as one week did not display haptoglobin, whereas animals more than a month old did (Shaughnessy, 1970). Laws listed two other phocids having large newborn young: the leopard seal, H. leptonyx, and the Weddell seal, L. weddelli. From these observations, one would also expect to find haptoglobins in the newborn young of these species.

Rather than crudely estimating the concentration of haptoglobin by classifying its staining intensity on starch gels, it would be more satisfactory to quantify it, using, for example, the rapid method outlined by Roy, Shaw and Connell (1969) which utilizes the fact that haptoglobin protects haemoglobin from acid denaturation.

Comparison of electrophoretic mobilities

Electrophoretic mobility of blood proteins has frequently been used as a taxonomic tool at supraspecific levels (e.g., Johnson, 1968; Clark, 1972). Information is available from this study on the haemoglobin pattern in five pinniped species representing three families: P. vitulina, H. fasciata and E. barbatus (Phocidae); E. jubata (Otariidae) and O. rosmarus (Odobenidae). The haptoglobin, haemopexin and transferrin patterns in the three phocids were also determined.

The three phocids studied here are members of two tribes of the

subfamily Phocinae as described by Burns and Fay (1970) (Phocini: P. vitulina, H. fasciata; Erignathini: E. barbatus). Haptoglobin and haemopexin have different mobilities in each of these species. Their mobilities in P. vitulina and H. fasciata are most alike which is consistent with the above classification. However, transferrin of E. barbatus and the common transferrin of P. vitulina have the same mobility, while those of H. fasciata are faster. Also, haemoglobin patterns are identical in the three species. Thus, electrophoretic data provided by two of these four proteins (haptoglobin and haemoglobin) are consistent with the accepted classification of this subfamily, but a classification based on either of the other proteins (transferrin and haemoglobin) could be misleading.

Seal, Erickson, Siniff and Hofman (1971) have shown that a classification of pinnipeds into subfamilies on the basis of the mobility of the slow haemoglobin zone in starch gels agrees with the currently accepted classification (see Fig. 20). They observed that the slow zone in four members of the Phocinae (Phoca, Pusa, Erignathus and Halichoerus) had a mobility of 0.30 relative to the common fast zone. In the four antarctic genera, Leptonychotes, Lobodon, Hydrurga and Ommatophoca (which they placed in the subfamily Lobodontidae [sic], but which are usually placed in the Monachinae, e.g., by Scheffer, 1958; King, 1964), the slow zone had a relative mobility of 0.75.

In this study, the relative mobility of the slow zone of Histriophoca (0.3) was the same as that of Phoca and Erignathus (Table 9). indicating that five of the phocines have the same haemoglobin pattern. This

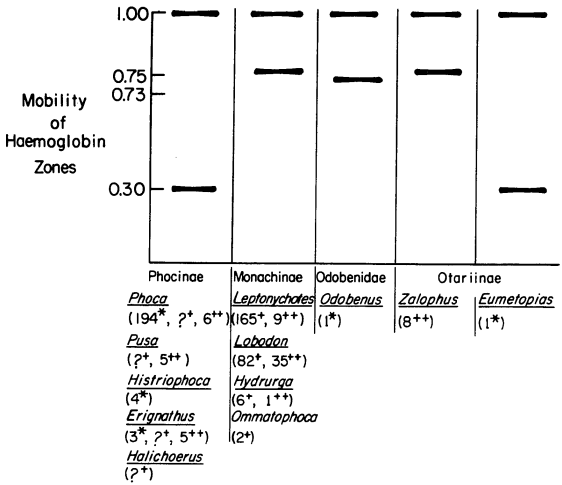

Figure 20. Mobility of haemoglobin zones of several pinniped genera as revealed by starch gel electrophoresis. The number of animals examined is given in parentheses. Data are from:

* This thesis.
\+ Seal, Erickson, Siniff and Hofman (1971).
++Seal (1969).

haemoglobin pattern of phocines should not be confused with that of the four phocines E. barbatus, P. hispida, C. cristata and P. groenlandicus reported by Naevdal (1966a; 1966b) and Suderman et al. (1973), in which haemoglobin appeared as an anodic major zone and a cathodic minor zone. The former pattern was observed by starch gel electrophoresis, whereas the latter was observed using electrophoresis on starch/agar gel, starch block and cellulose acetate.

The relative mobility of the slow zone of Odobenus observed in this study (0.7) is about the same as that of the antarctic phocids and Zalophus (Seal, 1969). However, the relative mobility of the slow zone in Eumetopias (0.3) is the same as that of the Phocinae. Thus, a classification of pinnipeds based on haemoglobin pattern is not consistent with the widely accepted morphological classification which puts Eumetopias, Zalophus and Odobenus in the superfamily Otarioidea and the phocines in the superfamily Phocoidea (e.g., Scheffer, 1958). It is also contrary to serological evidence based on albumins (Sarich, 1969b) and haemoglobins (Sarich, 1972) which indicates an even closer relationship between the Odobenidae (represented by Odobenus) and the Otariidae (represented here by Eumetopias and Zalophus), but could be construed as being consistent with the evidence from karyotypes which places the Odobenidae in an intermediate position between the Phocidae and the Otariidae (Fay, Rausch and Feltz, 1967). Thus, electrophoretic mobility of haemoglobin seems to be unreliable as a taxonomic tool at higher than the tribal level among pinnipeds.

APPENDIX I

ELECTROPHORETIC EXAMINATION OF BLOOD PROTEINS IN BELUGA WHALES

Cetaceans are divided into two Orders: the Mysticeti, containing the baleen or whalebone whales, and the Odontoceti, containing the toothed whales. Beluga whales (Delphinapterus leucas) are odontocetes of the family Monodontidae. The nomenclature used for cetaceans in this thesis follows that of Rice and Scheffer (1968).

1. Distribution of Beluga Whales

The distribution of belugas in the North Atlantic, North Pacific and Arctic Oceans has been discussed in detail by Kleinenberg et al. (1964). They reported that belugas in Bering Sea migrate northward in spring and summer and southward in winter, although some animals were known to occur year-round in Mechigmen Gulf (near Bering Strait) and in polynyas in the Chukchi Sea and the Canadian Arctic. Since insufficient data were available, they could not comment on beluga migrations near the Alaskan coast.

Tomilin (1957, 721) noted that large concentrations of belugas had been observed in the Gulf of Anadyr in summer. During freeze-up they were observed to head southeast toward the open sea, where they were believed to winter. He also pointed out that no data were available on the affinities of belugas from the Gulf of Anadyr and those from coastal Alaska. As indicated earlier (p. 6), beluga populations in Cook Inlet and Bristol Bay are thought to be resident, while herds from the western Canadian Arctic and eastern Siberian Arctic are believed to winter in central Bering Sea (Klinkhart, 1966; Fay, 1971; Mansfield, 1971).

Systematics of belugas have been studied by Sergeant and Brodie (1969). They examined body measurements of belugas from several arctic and subarctic localities, and interpreted non-overlapping differences in size in terms of separation of populations. In general, whales were larger in subarctic than in arctic localities. An exception was the population of small belugas in Bristol Bay which they thought might be the result of genetic mixing with small arctic whales that migrate through Bering Strait. No significant difference was observed between belugas from the eastern and western Canadian Arctic, separated by 400 km in Viscount Melville Sound, that the authors believed would not provide a barrier to beluga movements.

2. Previous Electrophoretic Studies of Cetaceans

Some studies on the biochemical identification of whale populations have been performed using immunological procedures (Cushing, 1964; Naevdal, 1971). Studies utilizing electrophoretic procedures have examined a few members of one or more species, for one or more of the proteins haemoglobin, haptoglobin, LDH or transferrin (Table 11). Due to insufficient data, there has been no attempt to use electrophoretic results to measure the amount of genetic variation within a species, nor to use the observed variation to identify populations.

Haemoglobin

Haemoglobin has been examined electrophoretically in many cetacean species. No intra-specific variation was observed, but it is unlikely that differences would be detected in such small series.

TABLE 11. A list of the cetaceans that have been examined electrophoretically.

Species	Number of cetaceans examined electrophoretically for Haemoglobin[1]	Haptoglobin[2]	LDH[3]	Transferrin[4]
Order Mysticeti				
Eschrichtius gibbosus	1	-	-	-
Balaenoptera borealis	2 or 5 (?)	2 or 5 (?)	5 or more	-
Balaenoptera physalus	16	16	-	-
Megaptera novaeangliae	8	8	✓	-
Order Odontoceti				
Platanista gangetica	1	-	-	-
Inia geoffrensis	3	-	-	-
Pontoporia blainvillei	4	-	-	-
Steno bredanensis	1	-	1	-
Tursiops truncatus	5	-	-	-
Tursiops gilli	3	-	-	-
Grampus griseus	1	-	1	1
Lagenorhynchus obliquidens	5	-	-	-
Stenella longirostris	3	-	-	-

TABLE 11. continued

Species	Number of cetaceans examined electrophoretically for			
	Haemoglobin[1]	Haptoglobin[2]	LDH[3]	Transferrin[4]
Stenella caeruleoalba	2	-	33	11
Delphinus delphis	5	-	1	11
Globicephala melaena	-	-	1	5
Globicephala macrorhyncha	5	-	-	-
Orcinus orca	6	-	-	-
Phocoena phocoena	1	-	-	-
Phocoenoides dalli	3	-	24*	-
Physeter catodon	2 or 5 (?)	2 or 5 (?)	√	-

√Number of animals not given.

*Including 20 of the truei color phase.

[1]Hovarth et al. (1968); de Monte and Pilleri (1971); Travis, Saunders and Cushing (1971); Baluda, Kulu and Sparkes (1972).

[2]All adults; 11 foetuses of unstated species examined also (Travis et al., 1971).

[3]Numachi (1970); Gallien, Chalumeau-le-Foulgoc and Fine (1970); Lyons and Erickson (1965), who also examined two unnamed mysticetes.

[4]Gallien et al. (1970).

Haptoglobin

Haptoglobin has been examined electrophoretically in four species by Travis et al. (1971). A single haptoglobin zone, with mobility similar to that of human haptoglobin Hp 1-1 was observed in 24 of the 31 adults examined, including an unstated number from each species. This zone was observed in reduced intensity in two of 11 foetuses, and presumably, was absent from the other nine.

Lactate dehydrogenase

LDH patterns have been reported for a total of 11 species. Numachi (1970) examined LDH in three odontocetes: S. caeruleoalba, P. dalli and S. bredanensis. Each animal possessed five zones, which had the same mobility in each species. Gallien, Chalumeau-le-Foulgoc and Fine (1970) performed a comparative study of the general serum protein pattern, transferrin mobility and LDH pattern of the four odontocetes: D. delphis, S. caeruleoalba, G. griseus and G. melaena. They examined the LDH pattern in four individuals (presumably one from each species), and found that each possessed five zones. Lyons and Erickson (1965) briefly reported on LDH in five species: four mysticetes (B. borealis, M. novaeangliae, and two unnamed species) and a single odontocete (P. catodon). In each case more zones than the usual five were observed. The authors attributed this to the presence of a third LDH monomer in each individual which resembled the M (also known as A) unit. These units, combined with the single H (or B) monomer, would result in the presence of 15 zones. Up to 13 zones were demonstrated, the others presumably being obscured.

Transferrin

Only two polymorphisms have been demonstrated electrophoretically in cetaceans, in both cases for transferrin (Gallien et al., 1970). Three transferrin genotypes were identified in D. delphis, and two in S. caeruleoalba by autoradiography. They also identified a single transferrin zone in G. melaena and G. griseus.

3. Materials and Methods

Serum samples were collected from 15 belugas at Elephant Point, Eschscholtz Bay (66° N, 161° W) in June 1971 during the harvest by Eskimos from Buckland, Kotzebue and other nearby villages. Animals were herded into shallow water on the north side of the bay by men in about 20 small boats powered by outboard motors, and then shot with rifles. It was not feasible to collect blood from cadavers in the water, due to the windy conditions. Once the animals were hauled into shallow water, butchering commenced, and blood samples were collected from the heart or great vessels. Care was necessary to ensure that sea water was not collected along with the blood.

Attempts to collect comparative series from Bristol Bay and Mackenzie Bay were unsuccessful, as was a request for material from the New York Aquarium.

Serum samples from belugas were examined for haptoglobin, haemopexin, LDH and transferrin, as described in Section IV. Although red cell haemolysates were prepared from only three animals, it was found that haemoglobin could also be examined electrophoretically in haemolysed

serum samples from another five animals.

4. Results

Haemoglobin

Haemoglobin of the eight animals examined consisted of a single zone with mobility 0.92 relative to human HbA (Fig. 21). The five animals in which haemoglobin was examined in haemolysed serum also displayed albumin zones.

Haptoglobin and haemopexin

Three peroxidase-active zones that migrated to the anode were observed in serum samples, or in serum to which beluga haemoglobin had been added (Fig. 22). By analogy with the situation in harbor seals, they were assumed to be methaemalbumin, haemopexin, and haptoglobin saturated with haemoglobin, in order of decreasing electrophoretic mobility. A fourth zone migrated toward the cathode, and was assumed to be free haemoglobin. Presumably another zone, representing unsaturated haptoglobin, could be demonstrated between haemopexin and saturated haptoglobin in unhaemolysed serum samples. Haptoglobin and haemopexin were clearly resolved by this technique; no variation was observed in the mobility of either zone in the 15 animals examined.

Lactate dehydrogenase

At least four LDH zones were observed migrating to the anode in each sample (Fig. 23). Although considerable quantitative variation was evident in LDH patterns of the 15 animals examined, differences in mobility were not apparent.

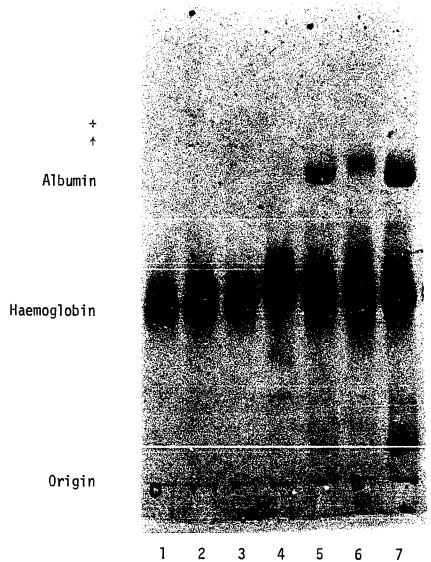

Figure 21. Acrylamide gel electrophoretogram of Delphinapterus leucas and human red cell haemolysates and haemolysed sera, showing haemoglobin zones. The samples are:

1. EP 3, red cell haemolysate.
2. EP 11, red cell haemolysate.
3. EP 13, red cell haemolysate.
4. Human, red cell haemolysate.
5. EP 12, haemolysed serum.
6. EP 15, haemolysed serum.
7. EP 18, haemolysed serum.

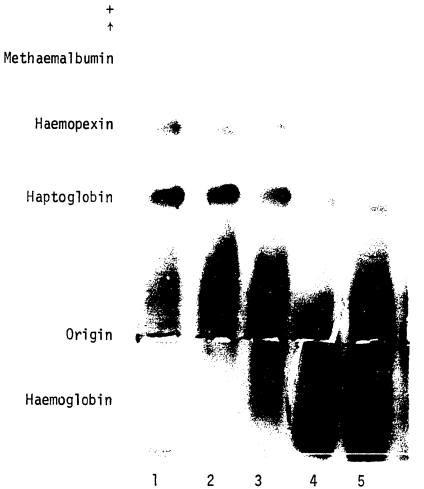

Figure 22. Starch gel electrophoretogram of Delphinapterus leucas sera, with added red cell haemolysate, showing peroxidase-active zones. The samples are:

1. EP 13.
2. EP 14.
3. EP 16.
4. EP 17.
5. EP 18.

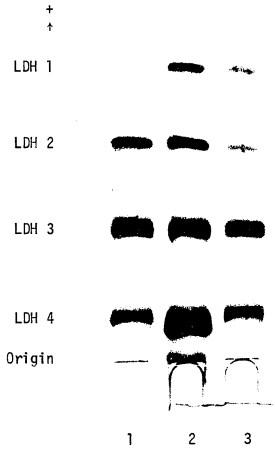

Figure 23. Acrylamide gel electrophoretogram of Delphinapterus leucas sera, showing LDH zones. The samples are:

1. EP 14.
2. EP 18.
3. EP 13.

LDH was examined also in serum samples from four Pacific bottle-nosed dolphins, T. truncatus, at Marine World, Redwood City, California. Four anodal zones were identified in each sample (Fig. 24). Differences in mobility were not apparent.

Transferrin

A pair of transferrin zones was detected by autoradiography, and was observed to migrate behind albumin on acrylamide gels (Fig.25 and 26). No variation was apparent in the mobility of transferrin in the 15 animals.

5. Discussion

The material from belugas was obtained from only one population, and there have been no other electrophoretic studies of beluga proteins with which to compare it. The results reported here will provide a base against which future studies of other beluga populations can be compared in order to test some of the proposals concerning beluga systematics and migrations outlined above.

There does not seem to be a pattern to the number of haemoglobin zones in cetaceans. Three of the Mysticeti listed in Table 11 possessed two haemoglobin zones, while only one zone was observed in E. gibbosus. Among the Odontoceti, two zones have been observed in 11 species (P. blainvillei, S. bredanensis, T. gilli, G. griseus, S. longirostris, S. caeruleoalba, D. delphis, G. macrorhyncha, O. orca, P. dalli and P. catodon), while only a single zone has been observed in another 5 species (P. gangetica, I. geoffrensis, T. truncatus, L. obliquidens

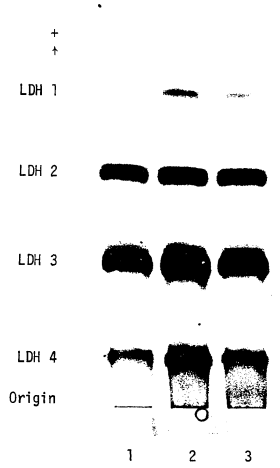

Figure 24. Acrylamide gel electrophoretogram of Tursiops truncatus sera, showing LDH zones. The samples are:

1. Tursiops 1.
2. Tursiops 2.
3. Tursiops 3.

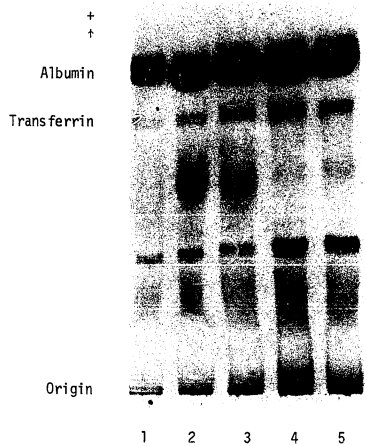

Figure 25. Acrylamide gel electrophoretogram of Delphinapterus leucas sera, showing transferrin zones. The samples are:

1. EP 17.
2. EP 11.
3. EP 10.
4. EP 3.
5. EP 2.

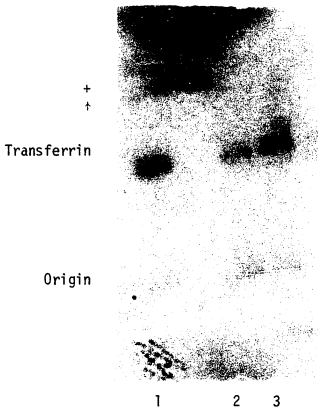

Figure 26. Autoradiograph of Delphinapterus leucas serum with added Fe^{59}, showing transferrin zones. The samples are:

1. EP 3.
2. EP 10.
3. EP 11.

and P. phocoena). To the latter must be added D. leucas from this study, also with one haemoglobin zone. Thus grouping cetaceans according to the number of haemoglobin zones does not produce the same dichotomy as the presently accepted concept of two orders.

The report of a third LDH monomer in four mysticetes and an odontocete (P. catodon) by Lyons and Erickson (1965) could indicate that all of these animals were heterozygotes. The probability that an individual is a heterozygote in a panmictic population without selection is, at most, 0.5. Since these authors indicated that at least five B. borealis were examined, it is unlikely that they were all heterozygotes ($P<0.03$). An alternative interpretation is that the gene controlling the M (or A) unit has duplicated, so that three structural gene loci control the synthesis of LDH in these five species, rather than the usual two. Since several odontocetes other than P. catodon possess only five LDH zones (including D. leucas and T. truncatus examined in this study), if gene duplication is the cause of the proliferation of LDH zones observed by Lyons and Erickson (1965), it must have occurred independently in each of the odontocete and mysticete phyletic lines after their divergence.

In humans, transferrin migrates as a β-globulin (Poulik and Smithies, 1958). In two of four odontocetes examined by Gallien et al. (1970), namely D. delphis and S. caeruleoalba, transferrin had a much faster mobility than human transferrin, and in the other two species (G. griseus and G. melaena) it was slightly faster than human transferrin. To these odontocetes with rapidly-migrating transferrin must now be

added D. leucas. Gallien et al. (1970) pointed out that such transferrin zones had been observed previously only in Equidae and some fishes. The reason for rapidly-migrating transferrins in odontocetes is not known.

APPENDIX II

ELECTROPHORETIC EXAMINATION OF BLOOD PROTEINS IN POLAR BEARS AND GRIZZLY BEARS

1. Distributions and Taxonomy

The polar bear (Ursus maritimus) and grizzly (or brown) bear (U. arctos) are large carnivores with northern distributions: the former circumpolar about the Arctic Ocean, on the ice and on adjacent coasts and islands, and the latter in North America, mainly north of 50° N (Rausch, 1963). Considerable attention has recently been focused on conservation and biological studies of polar bears (e.g., Lentfer and Brooks, 1970), and on the possible conflict between grizzly bears and the oil industry in northern Alaska (Weeden and Klein, 1971).

Population affinities of polar bears are currently being examined by Larsen (1971) by an electrophoretic examination of blood and milk proteins, and have recently been investigated by Manning (1971), utilizing variation in cranial characters. The latter author examined 628 skulls from five regions (Spitzbergen, east Greenland, west Greenland - Canada, "Alaska south" (= west ?), and "Alaska north") and demonstrated the existence of a cline of increasing skull size from east Greenland westward toward Bering Strait. The two Alaskan regions were designated by a line running northwest from Point Lay (on the suggestion of J. W. Lentfer). Elsewhere, Lentfer (1971) has briefly noted differences in skull and body size of polar bears from the north and west coasts of Alaska. Although Manning could not suggest any current geographical barrier, he suggested that the two Alaskan populations might have been separated by the Bering Land Bridge during the Pleistocene glacial periods and only recently become contiguous.

An attempt to identify populations of brown bears in North America was made by Rausch (1963), who considered only skulls that had attained maximum length as indicated by closure of the basioccipital-basisphenoid suture. A comparison of condylobasal length in 27 series comprising 357 skulls showed clinal variation, but the wide range of individual variation prevented characterization of local populations, except for those from Kodiak, Afognak and Shuyak Islands (U. a. middendorffi), which he considered to be reproductively isolated and subspecifically distinct from those of the remainder of North America (U. a. horribilis). Further, he considered that the plethora of species and subspecies recognized by Merriam (1918) was not justified.

In this study, LDH and transferrin were examined electrophoretically in series from the two Alaskan populations of polar bears and an Alaskan population of grizzly bears.

2. Materials and Methods

Blood samples from 46 polar bears were provided by Mr. J. W. Lentfer, Alaska Department of Fish and Game, for Mr. W. A. Galster of the Institute of Arctic Biology. These samples were collected from freshly killed animals by hunting guides operating out of Barrow (22 samples: 11 of serum and 11 of red cells) and Kotzebue (24 samples: 13 of serum and 11 of red cells).

Serum samples were obtained from 19 immobilized grizzly bears, four to 20 years old, on the North Slope of the Brooks Range between 138° W and 158° W by Mr. L. Crook, Alaska Department of Fish and Game.

LDH was examined by acrylamide gel electrophoresis in serum samples of grizzly bears, and in both serum and red cell samples of polar bears. The red cell samples were diluted with two volumes of sucrose solution before electrophoresis. Serum samples of both species were examined by acrylamide gel electrophoresis for transferrin, which was identified by autoradiography.

3. Results

Five LDH zones, migrating towards the anode, were visible in the 19 grizzly bears. Forty-three of the polar bears displayed an identical pattern. Three other polar bears (K 10975, B 13294 and B 13538) displayed another LDH phenotype, in which zones LDH 2, 3, 4 and 5 migrated faster than the corresponding zones in other bears (Fig. 27).

Each animal of both species possessed two transferrin zones that migrated towards the anode: a major zone and a faster minor zone (Fig. 28). These zones bound radioactive iron. Since the intensely-staining zone that migrated ahead of transferrin in some animals did not bind radioactive iron, it was not transferrin. Transferrin had the same mobility in all animals of both species.

4. Discussion

The polar bears examined in this study were polymorphic for an M chain variant of LDH. Since Shaw and Barto (1963) have demonstrated the presence of 15 LDH zones in heterozygotes and five in homozygotes, the three variant polar bears (each with five zones) must be homozygotes.

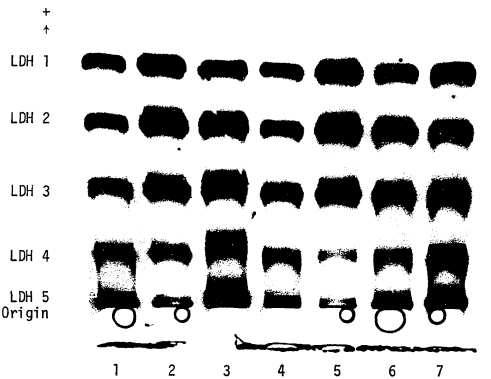

Figure 27. Acrylamide gel electrophoretogram of Ursus maritimus and U. arctos horribilis sera, showing LDH zones. The samples are:

1. U. maritimus K10977.
2. U. a. horribilis 3017.
3. U. maritimus B13294.
4. U. maritimus K10957.
5. U. a. horribilis 3018.
6. U. maritimus K10941.
7. U. maritimus B13504.

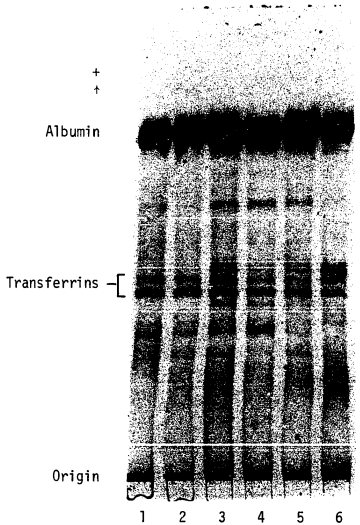

Figure 28. Acrylamide gel electrophoretogram of Ursus maritimus and U. arctos horribilis sera, showing transferrin zones. The samples are:

1. U. a. horribilis 3010.
2. U. a. horribilis 3011.
3. U. maritimus K10638.
4. U. maritimus K10644.
5. U. maritimus K10629.
6. U. a. horribilis 3008.

However, the occurrences of two homozygous variants in a series of 22 homozygotes (that from Barrow), and one homozygous variant in a series of 24 homozygotes (that from Kotzebue), are unlikely events in panmictic populations without selection (P = 0.004 and P = 0.042, respectively). Thus, these observations might indicate that neither of the regions "Alaska north" and "Alaska south" (as recognized by Manning, 1971) currently contains a single breeding population.

No differences were observed in the transferrin electrophoretic pattern of the two species. Biochemical uniformity of some other ursid blood proteins (in particular those of polar bear and grizzly bear) has been demonstrated in earlier studies: by an electrophoretic investigation of haemoglobins (Seal, Swaim and Erickson, 1967; Seal, 1969); by tryptic peptide maps of haemoglobin (Seal, 1969); and by an immunological study of serum albumins (Seal, Phillips and Erickson, 1970). Thus, the variant LDH phenotype that occurred in a small proportion of polar bears is the only demonstrated difference between blood proteins of the two species.

CONCLUDING REMARKS

Biochemical identification of populations using the electrophoretic mobility of proteins has been used successfully in many vertebrate populations. The number of proteins involved in such studies has varied widely, from as few as one or two in, for example, the pinnipeds L. weddelli (Shaughnessy, 1969) and Arctocephalus sp. (Shaughnessy, 1970), up to as many as 36 in the house mouse, M. musculus (Selander et al., 1969). This last study, concerning mice from six geographic regions of Denmark, provides a splendid example of electrophoretic results separating populations into groups identical with the subspecific separation (M. m. musculus and M. m. domesticus) based on morphological characters. Similar uses of electrophoretic investigations at the species level have been made. For example, electrophoretic examination of proteins encoded by 23 loci in cotton rats, Sigmodon sp. by Johnson et al. (1972) supported earlier conclusions from karyological studies that two allopatric species, S. hispidus sensu stricto and S. arizonae, had previously been confused by morphological taxonomists. Since the electrophoretic approach has not been successful in this study in distinguishing harbor seal populations that have been separated on morphological, behavioral and ecological grounds, electrophoresis of blood proteins cannot be considered as a diagnostic criterion for all species, at least when based on a relatively small number of proteins. A similar lack of success was reported by Naevdal (1966a; 1969) in a study of harp seals, P. groenlandicus from eastern

Canada, Jan Mayen and the White Sea. They had previously been described as three isolated breeding populations on the basis of cranial characters (Yablokov and Sergeant, 1963) and color patterns (Yablokov and Etin, 1965), but Naevdal found that haemoglobin had identical mobility in each population, and that, although transferrin was polymorphic, differences in allele f requency were not significant. However, in this case, even fewer proteins (two) were examined than in the present study.

Conversely, the study of a small number of polar bear blood proteins reported in this thesis indicates that electrophoretic characters can be more sensitive than morphological characters for population identification when an appropriate protein is found.

Mobility of proteins determined by gel electrophoresis has freqently been used as a taxonomic tool at supraspecific levels, e.g., by Sibley and Hendrickson (1970) in birds, and Johnson (1968) in mammals. However, the approach is generally considered to be more pertinent at the specific and population levels (e.g., Weiss and Goodman, 1971), because comparative electrophoretic studies at the higher taxonomic levels are confused by the presence of polymorphisms and the possibility that small changes in the amino acid composition of a protein can drastically alter its charge and hence its electrophoretic mobility. Results obtained in this study from electrophoresis of pinniped haemoglobins and cetacean LDH, considered with reports of other electrophoretic studies on these animals, reinforce the belief that conclusions concerning supraspecific affinities based on the electrophoretic mobility of proteins contradict the classical classification so frequently as to render them unreliable.

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